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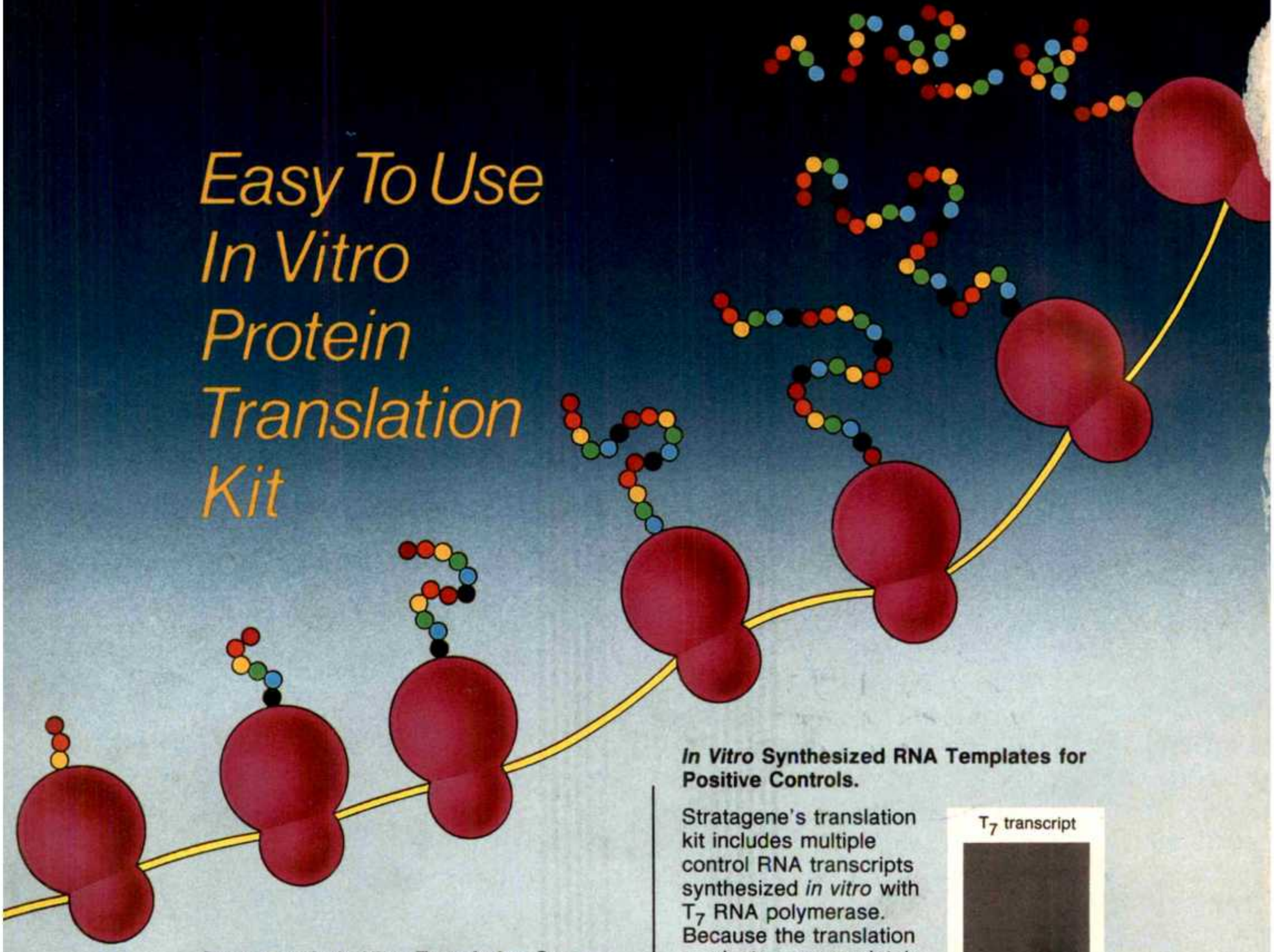
WATER ON MARS

SPECTROSCOPY/PITTSBURGH
product review



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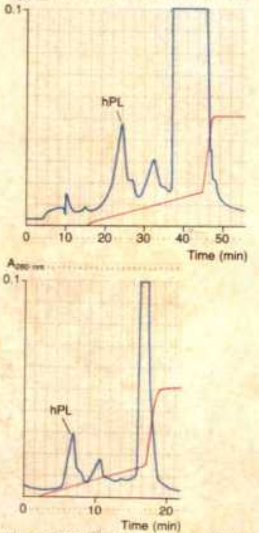

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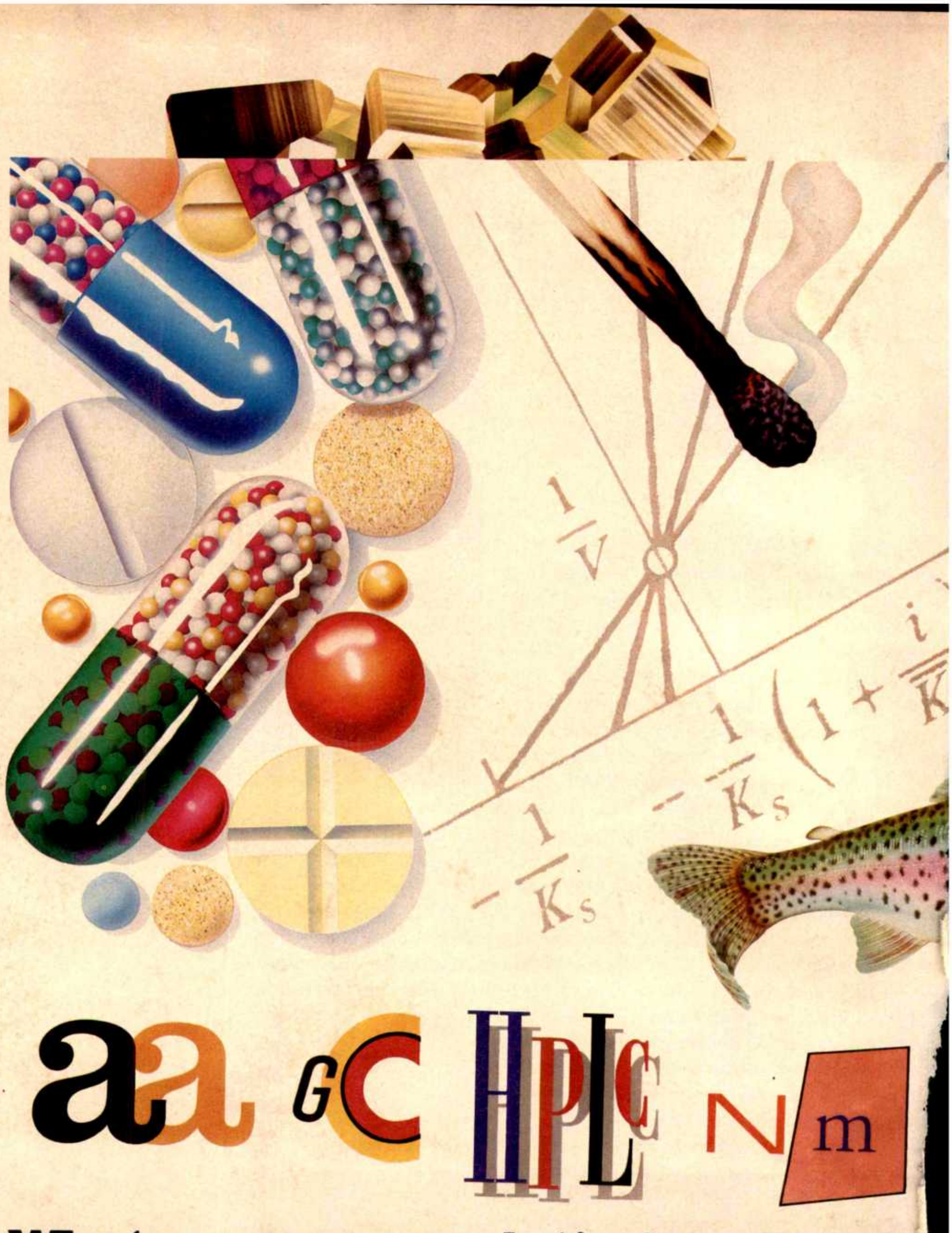

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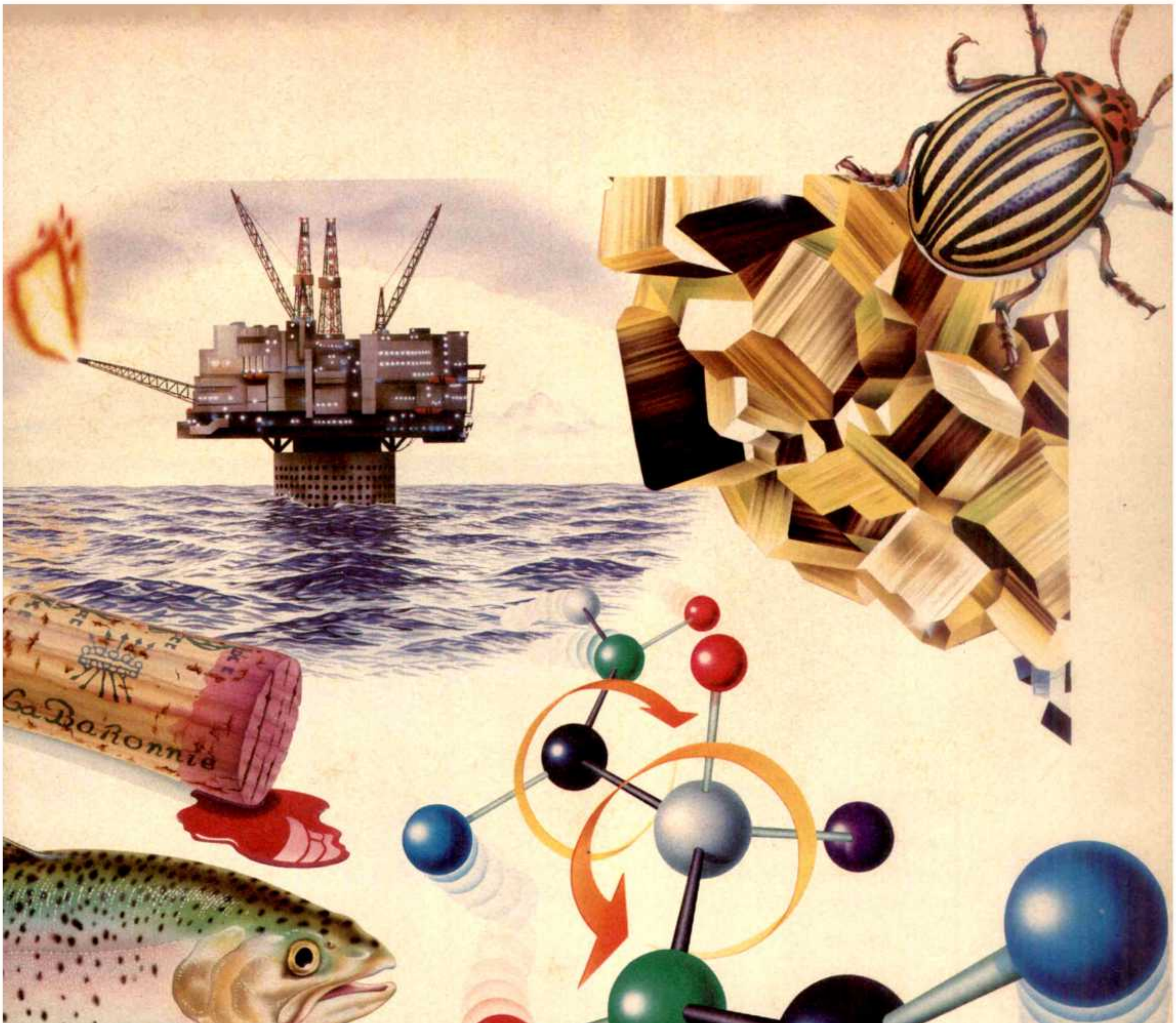

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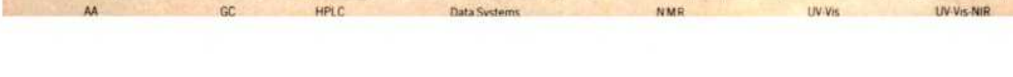

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5 March 1987
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A computer-generated mosaic of part of the northern hemisphere of Mars, made by merging 102 Viking Orbiter frames taken in the red and violet with a synthetic green filter image made by interpolation. At the bottom is the equatorial canyon system. Dark spots on the left are the large Tharsis volcanoes (A. McEwen/USGS-Flagstaff). See page 30

THIS WEEK

■ Through the ozone hole

. . . pages 19 and 59

Balloon-borne ozone sensors show that the hole in the ozone layer that develops in September and October in the Antarctic stratosphere has some remarkable features that cannot be accounted for by existing theories.

■ Magma chamber

. . . pages 15 and 35

Seismic profiles provide the best evidence yet of a permanent chamber of molten rock under the ocean ridges, where the plates of the Earth spread apart a few centimetres a year.

■ Neolithic Dutch elm disease

. . . page 72

Neolithic fossil pollen suggests that the decline of elm trees in Europe some 5,000 years ago may have been caused by Dutch elm disease.

■ A larger, slower Sun

. . . page 52

Recordings made by French astronomers between 1666 and 1719 imply that during the 'Little Ice Age' of the seventeenth century, the Sun was larger, but rotated more slowly, than at present.

■ Cleft palate gene

. . . page 91

Localization of a defective gene responsible for the cleft palate in a large Icelandic family to a small segment of the X chromosome is a starting point for the identification of the gene and an analysis of the genetic, as opposed to the environmental, causes of the cleft palate.

■ Positive darwinism

. . . pages 12 and 96

In the serine proteinase inhibitor group of proteins, the protein sequence of the reactive centre undergoes more rapid evolution than the rest of the molecule.

■ Annual index

. . . out now

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1986 Index
Volumes 319-324

The 1986 *Nature* subject and author index is now published. To order a copy see details on the second page of contents. Technical problems have regrettably meant that no author indexes were published for the last two volumes of 1986, but the information is included in the Annual Index; publication of bimonthly author indexes will resume for 1987.

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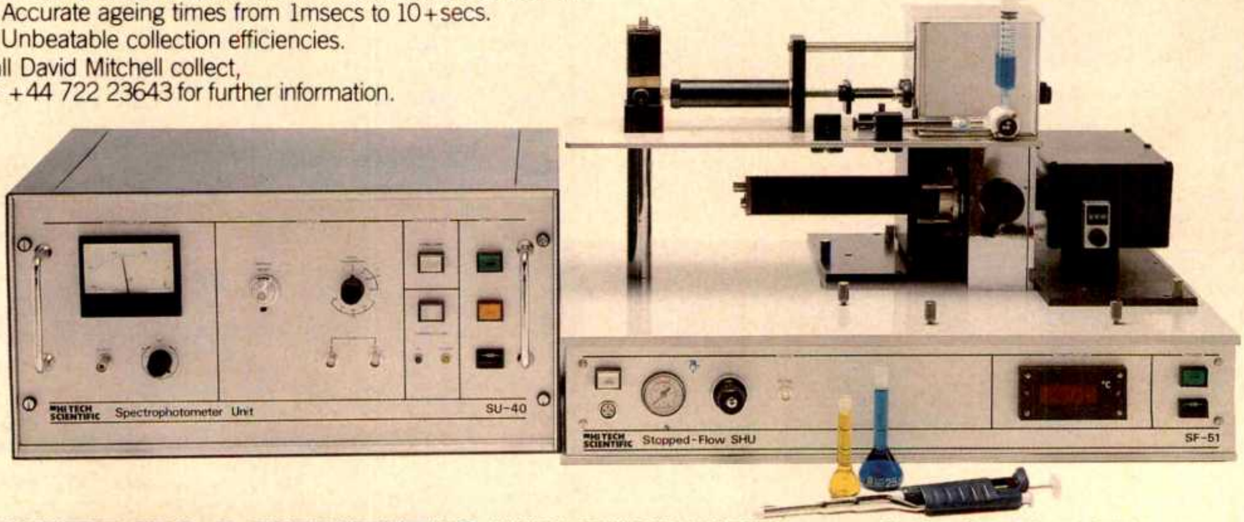
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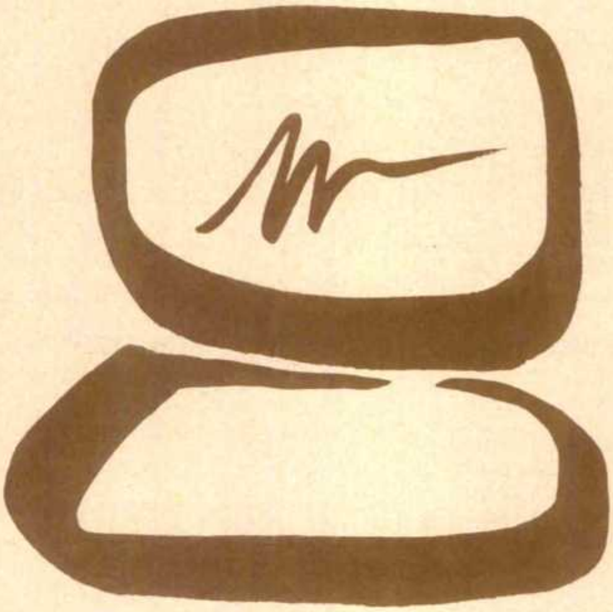

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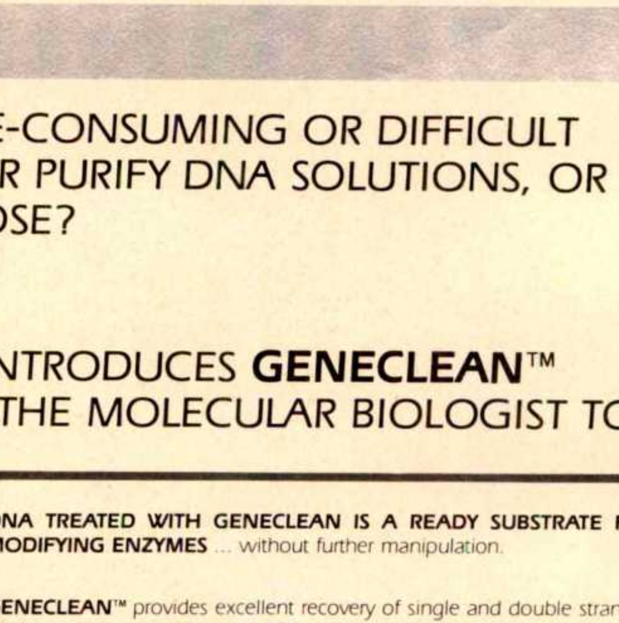
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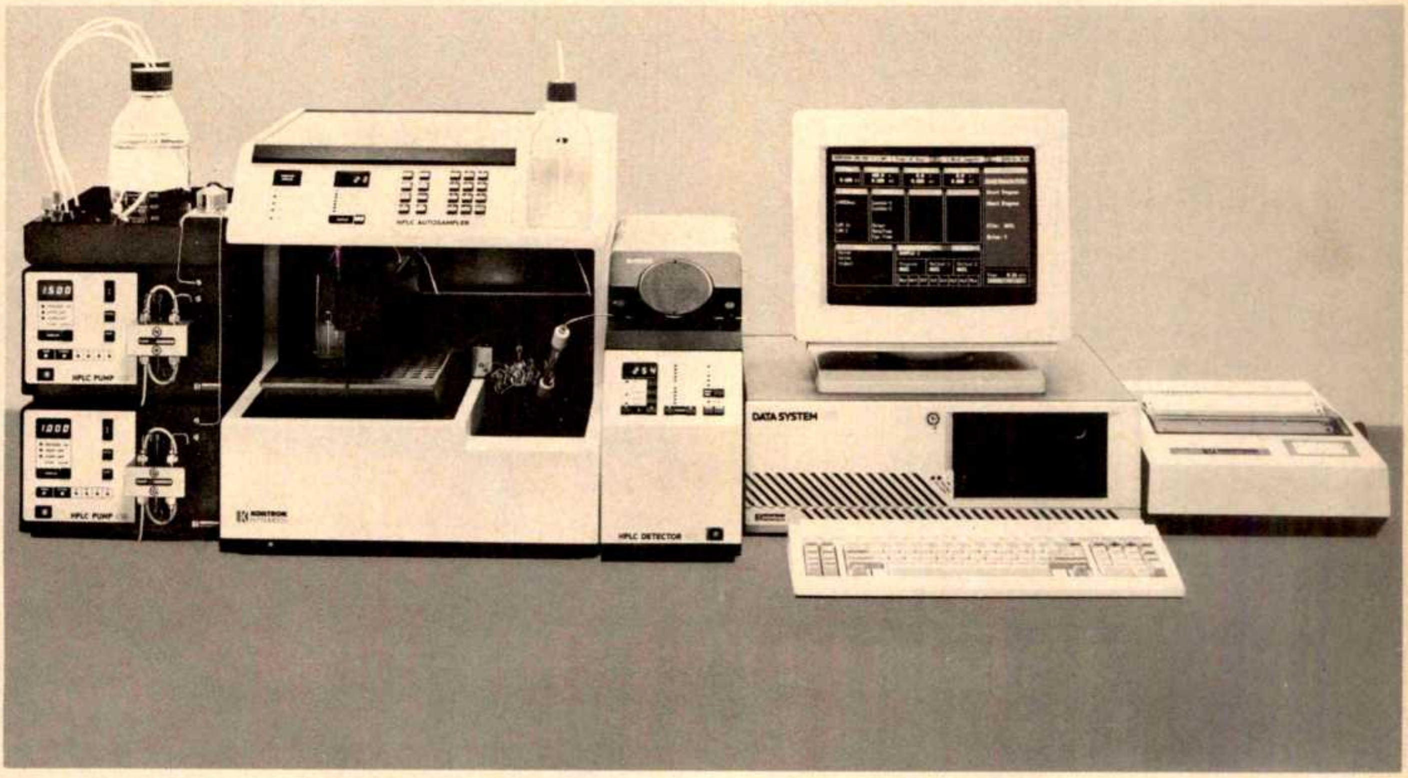

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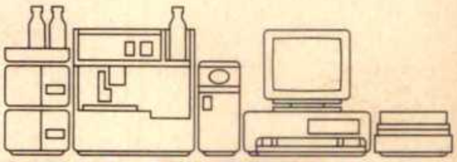

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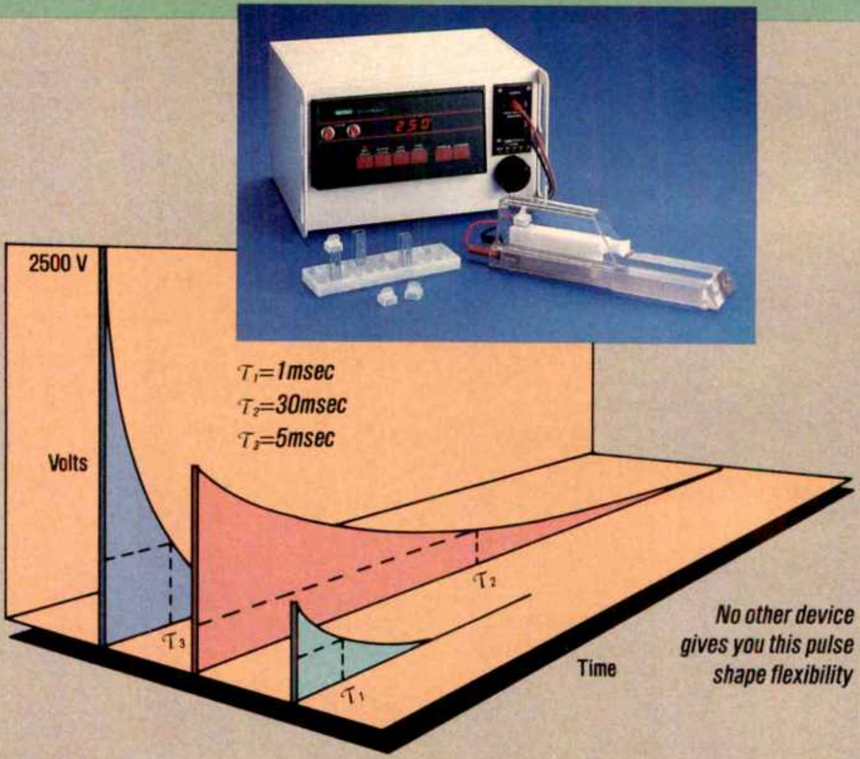

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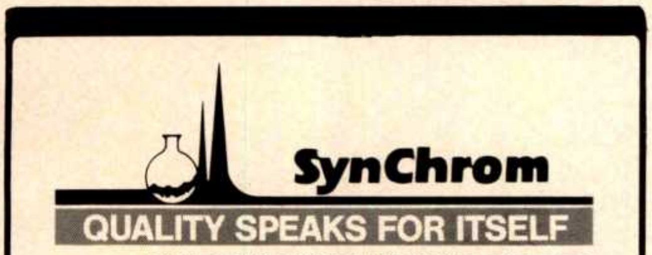

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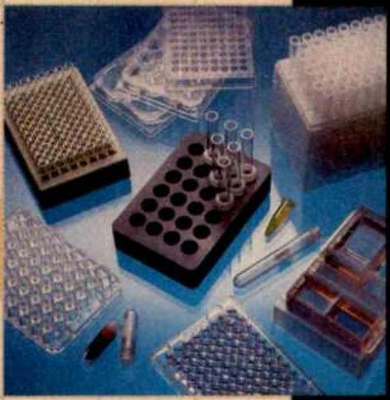

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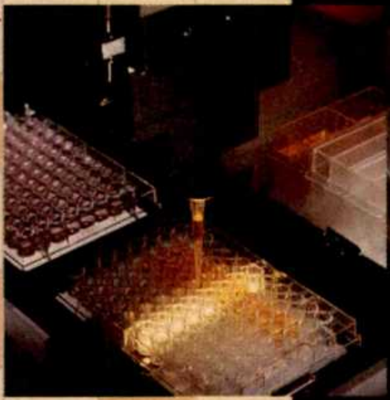

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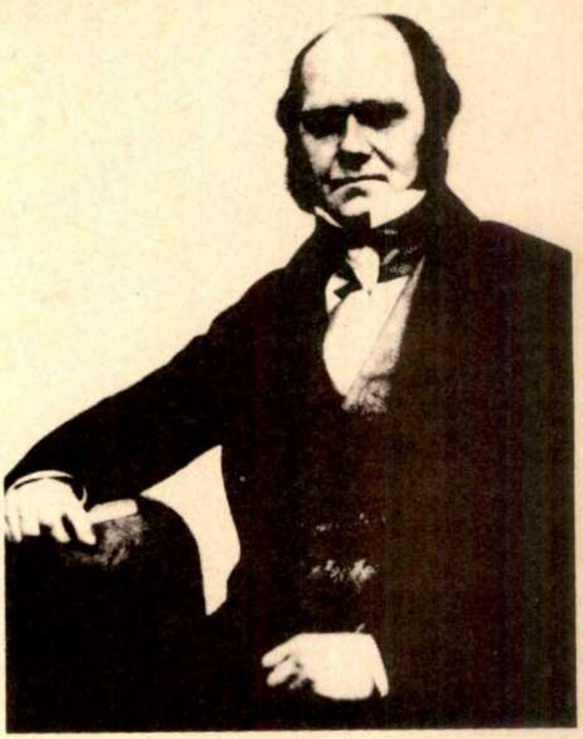

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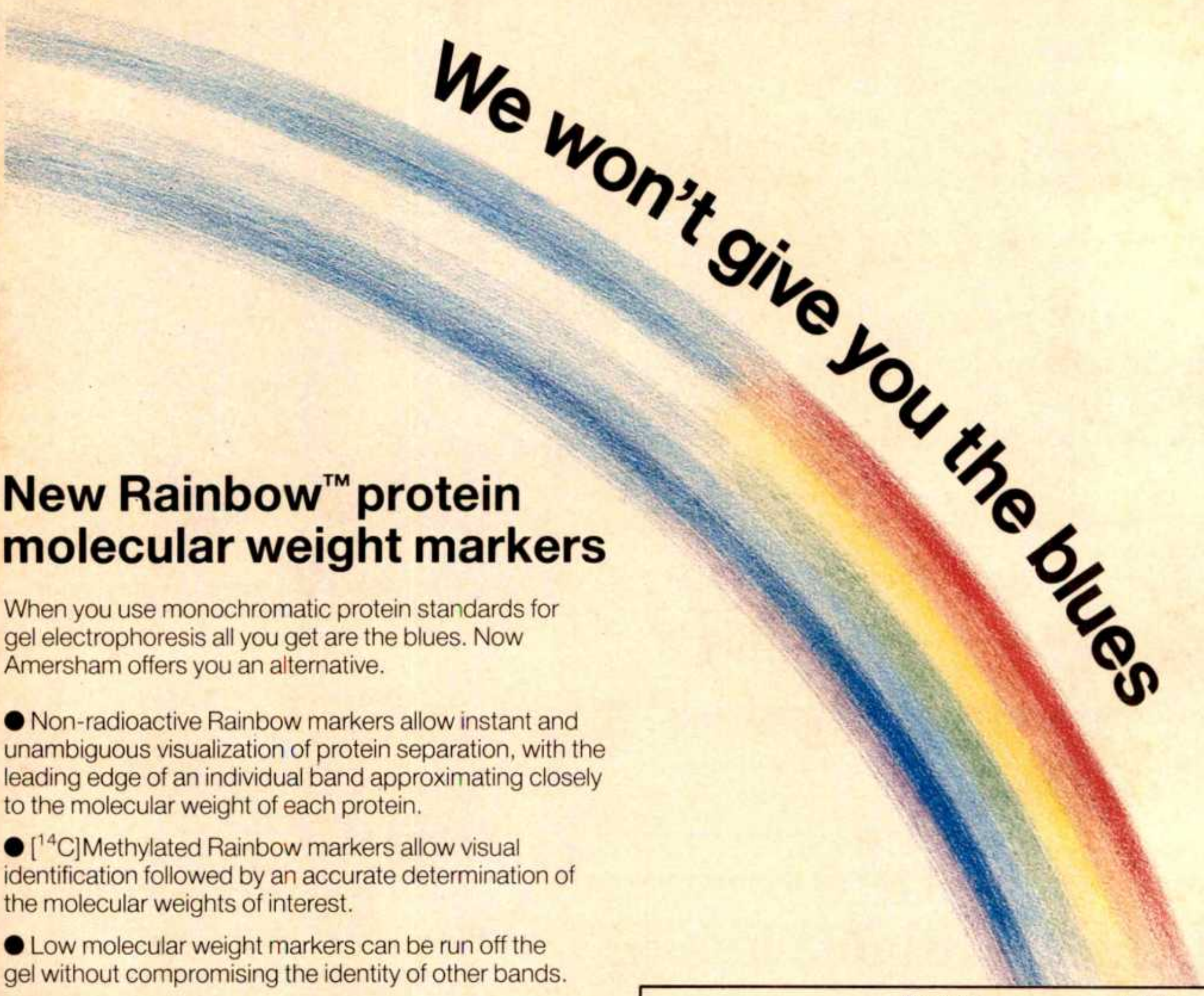
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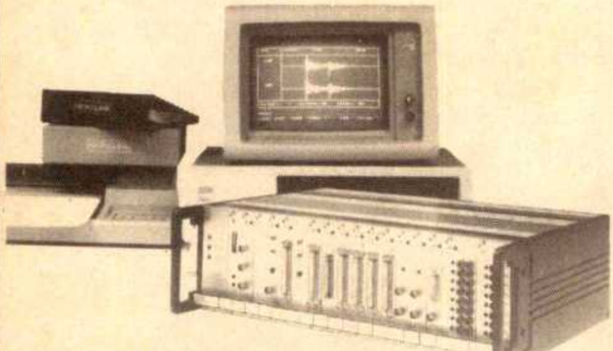

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nature

Back to Reykjavik (or 1979)

The new Soviet proposals on European missiles have been welcomed (as they should have been) even though they imply no more than a return to the late 1970s.

Mr Mikhail Gorbachev's proposal, last weekend, that there should be an immediate agreement to get rid of missiles of intermediate range in Europe is unexpected but welcome. For practical purposes, it is the 'zero option' first suggested three years ago by the United States which was one of the ingredients of the package abortively canvassed during last November's breathtaking and abortive summit meeting at Reykjavik. What has changed, since last November, is that Gorbachev is no longer asking that an agreement on intermediate missiles in Europe should wait on agreements on strategic arms and on the ambition of the US president to build a defensive shield against nuclear weapons. It seems a big step forward, although it will be easier to be sure when the negotiators at Geneva have had a chance this week to read the fine print. Meanwhile, there are good reasons why everybody should realize that what is now proposed is a return to the military disposition of nuclear weapons that obtained in Europe in 1979. For Europeans, at least, that was a more comfortable world, but it was in no sense a world free from trouble.

It is also proper, if out of spirit with Gorbachev's evident wish that something should be done to lessen military tensions, to recall that the deployment of US missiles in Western Europe is a direct consequence of the earlier deployment of SS-20 missiles by the Soviet Union. Neither of these developments had been foreseen just a few years earlier, during the negotiation of the SALT II treaty on the regulation of strategic missiles. The unannounced appearance of some hundreds of mobile SS-20s capable of launching nuclear warheads at any target in Western Europe cannot but have engendered a mixture of fright and indignation among governments in Western Europe; by itself, the deployment of the Soviet missiles would have ensured that the SALT II Treaty would not have been ratified by the Senate of the United States (but there were many other impediments to that course in 1979). The practical consequence has been the deployment by the United States of a countervailing force of cruise and Pershing II missiles on the territory of half a dozen West European countries. By agreeing to uncouple consideration of an agreement on intermediate missiles from the other issues on the agenda of the bilateral negotiations at Geneva, Gorbachev has tacitly agreed to roll back these uncomfortable pages of history.

That seems to be the spirit of the chorus of welcome, tinged with idiosyncratic caution, for Gorbachev's proposal. Dr Hans Dietrich Genscher, the Free Democrat foreign minister in the West German coalition government, was characteristically quick to throw his hat in the air in celebration. The statement from Lord Carrington, the secretary-general of the North Atlantic Treaty Organization, that the proposal is a "step forward" will carry great weight, as will that attributed to Mr Richard Perle, the Pentagon's professional sceptic on arms control, that Gorbachev's proposal is "promising". Why are so many people so welcoming? There are some practical reasons; the Netherlands, for example, which is committed to deploy US cruise missiles next year, will be glad if it can be relieved of that politically contentious obligation. Mr Neil Kinnock, the leader of the British Labour Party committed by last October's annual conference to a policy of unilateral nuclear disarmament may conversely be hoping that an agreement on intermediate missiles will make his policy less of an electoral liability. But the truth is that Gorbachev has timed his proposal well. After the disappointments of the past few weeks, the US president is likely to be more receptive than for months to an agreement such as that now on the table. More than that, after two years of negotiation that seems to have made very little headway, and with the wrangle about the compatibility of the Strategic Defense Initiative and the ABM Treaty threatening to become more bitter, the electorates of the West would like to see some action. So too, no doubt, would Gorbachev's constituents.

That is why a full-throated welcome for the Soviet proposal should be blended with an appreciation of the problems that would survive a deal. The technical objection to both sides' Euromissiles is the ambiguity of their military function. Although not formally classified as strategic, both forces could be used strategically, by being vehicles of threatened attacks on the other sides' cities, for example. At the extreme, they could conceivably (but dangerously) be used to call the supposed bluff of superpowers that have declared their willingness to assist more vulnerable allies by risking their own survival in a nuclear exchange. The potential removal of that risk is an important benefit of Gorbachev's proposal, but other problems of the same kind would remain. One is that both superpowers have scattered Europe with a variety of nuclear weapons designed for use in land battles, all of which could serve to escalate a conflict under way. Nuclear weapons carried by aircraft, of which there are some thousands within striking distance of strategically important European targets, are equivalent to, and as dangerous as, those carried by intermediate missiles. And then there are the British and French nuclear forces, explicitly designed to exert a strategic influence on the course of largely European conflicts. All these ways of making nuclear warfare would survive the acceptance of Gorbachev's proposals.

None of this implies that the game is not worth the candle. Rather, the lesson to be learned is that, in this part of the arms control canvas as elsewhere, no single agreement can be expected to stand on its own for the rest of time. So much is clear from the clutch of apparently hopeful treaties left over from the 1970s, all of which (with the exception of the partial test-ban treaty limiting underground tests of nuclear weapons to the equivalent of 150,000 tonnes of TNT) are being eaten away at the edges by the progress of military technology and undermined by their conceptual isolation. What the erosion of those old treaties shows is that even the most purposeful agreement will not last indefinitely unless it becomes a stepping-stone to other things, most simply another agreement, less tangibly, a better international climate. On this occasion, mercifully, Gorbachev has acknowledged that something will have to be done about battlefield weapons if an agreement can be reached on Euromissiles. What may surprise him is that with his decision not to make such an agreement hostage to agreements on strategic missiles and on ballistic missile defences, he will have improved the chances also of reaching agreements on those still more serious matters. □

Debtors call the tune

Brazil's decision not to pay interest on its debts will undermine the stability of the dollar.

By one of those little ironies, Brazil's announcement two weeks ago that it was suspending interest payments on its overseas debts immediately preceded the meeting of the finance ministers of the six rich countries of the West which declared that the value of the US dollar had declined sufficiently in the previous eighteen months, and that they would henceforth use their influence to keep it more or less where it is at present. In reality the two events are linked, are contradictory and, for what it is worth, full of gloomy portents. This is why.

Brazil owes the rest of the world $100,000 million in round numbers, much of it dating back to between six and fifteen years ago when the commercial banks of the West were awash with US dollars deposited by oil-producing states neither able nor willing to spend the money on goods. The banks were able to hold the deposits profitably only by lending them to others willing to pay interest on their borrowings; Brazil and similarly industrializing states appeared to be prime candidates. But by the beginning of this decade, the bankers had began to worry. The sheer volume of international debt had become too large for comfort. Then it emerged that one of the potentially most powerful sources of loans — the economy of the United States — had switched almost overnight from being a lender to a borrower.

The result has been entirely predictable. For the past five years, the reputation of the US economy for flexibility and the size of the federal deficit has kept US interest rates high and the dollar 'strong'. If the propensity of US citizens to save had been greater, the Reagan administration's aversion to tax increases would not have mattered, for the deficit would have been covered (as is the proportionately greater deficit of the government of Japan) by people's willingness to forgo consumption. In the event, people (mostly represented by financial institutions) elsewhere have helped balance the books by buying dollar securities with their savings, accumulating in the process substantial assets, chiefly land, buildings and industrial plant, in the United States. The US trade deficit ($180,000 million last year) is the counterpart of this process, an arithmetical consequence of the inward flow of capital.

This has been a frightening period for the Brazils of this world, of which there are a great many. High interest rates have increased the cost of debt. Last year, Brazil paid $12,000 million in interest, roughly 4.5 per cent of its national income. During the same period, the commercial banks of the West have ceased to be the prolific sources of funds they were a decade earlier. Their new-found caution is understandable; as they see it, they would be imprudent to throw good money after bad. The snag is that international institutions such as the International Monetary Fund are incapable of lending on the scale required to help Brazil and the others because their member governments are no longer prepared to equip them for the task, and are in any case not able to raise the funds with which to do so. The decline in the value of the US dollar since the beginning of last year will serve only to exacerbate the problems of the developing industrializing countries, which will find it harder to sell their manufactures on the international markets.

Plainly, this state of affairs cannot continue much longer. The three generic classes which have connived in the past in the creation of these contradictions have an interest somehow to conspire to resolve them. If the banks begin to fall like ninepins because they are forced to acknowledge that the debts that others owe them are worthless assets, there will be an economic crisis. But there are no sanctions that can be applied against sovereign debtors that will not make matters worse. In these circumstances, declarations about the maintenance of the US dollar at some predetermined value cannot carry much weight. It might be different if there were some leeway in the system, such as might come if the savings ratio in the United States were greater, or if the budget deficit were less (which could not be brought about overnight without precipitating recession). The best solution would be for the still-rich countries of the world to refinance the international lending institutions at a decent level, even at the cost of the measure of domestic inflation this would cause for some of them. Otherwise nature will take its course. □

What human frontiers?

The Japanese government has some way to go in the definition of its basic biology programme.

The government of Japan has come a long way in the past year in its definition of its Human Frontiers project, first made public in a fog of inexactitude. By a distinctively Japanese process of consultation and iteration, what seemed at first to be a vague programme of research is slowly turning into a programme of basic biological research that could be more than merely worthwhile (see page 8). It is especially intriguing that, on one view at least, Japan's financial contribution to the project would be that of a source of support for research projects arising not only in Japan but elsewhere. It is high time some government broke the mould of the pretence that the pursuit of excellence in research is possible only in its own laboratories. It will be interesting to see what elements of this particular proposal survive in the final prospectus.

It will also be important to see how distinctively Japanese the final plan will prove to be. The grand objective is to design a stimulus for basic biological research by means of a fund estimated at $250 million a year, rather less than 5 per cent of what is spent each year in the United States by the National Institutes of Health alone. So the question for the planners is not only whether it is possible to define a set of objectives that will give the programme a distinctive sense of purpose but also that of accomplishing something memorable with comparatively modest funds. The question is especially teasing because the government is apparently hoping to use the programme as a means of killing off the legend that Japan lets others concentrate on basic research while bending the energies of its own people towards practical (and profitable) objectives. The diffidence is taken too far (and would be entirely beside the point if there were more support for research in Japanese universities). Meanwhile, with such a relatively moderate budget, the Human Frontiers Project should put diffidence aside and give the international quest for an understanding of life and its abberations a Japanese flavour.

What might that be like? The obvious temptation is to guess that, because Japanese companies have a splendid reputation in the design and manufacture of instruments, their energies should be harnessed under the rubric of the Human Frontier Science Program to schemes such as that for the sequencing of the human genome, in which instrumentation is bound to play an important part (see Wada, A., *Nature* **325**, 771;1987). People elsewhere should therefore understand that such a view overlooks the great value of the classical molecular biology by which a growing number of Japanese scientists are distinguished, not to mention the fields such as veterinary medicine in which Japanese scientists have won international acclaim. The Japanese community will be understandably resentful if, in the process of consulting others about new directions, it finds itself typecast as an offshoot of, say, the successful watchmaking industry in Japan. But by the same test, it would be unwise to overlook the opportunity for exploiting the ingenuity of Japan's existing army of skilled designers in the solution of problems in biology which do not normally come their way. Perhaps the best balance between proper resentment of being typecast and the understandable wish to make a mark will be to concentrate the resources of the Human Frontier Science Program on some field now languishing for lack of sufficient ingenuity. Why not ask "How does the brain work?" and see where that leads? □

AIDS arrives in Soviet Union — (Official)

• Health authorities issue warnings to people
• Scientists seek research links with West

London

The Soviet Union has made a U-turn on its attitude to AIDS (acquired immune deficiency syndrome). After several years of castigating it as a disease of decadent capitalism — and, on occasion, attributing its origin to US bacteriological warfare experiments that went wrong — the Soviet media have begun publishing interviews with high-level health officials setting out how to avoid contamination. Immediate measures include the establishment of an anonymous AIDS screening facility at Moscow's No. 2 Clinical Infection Hospital, and, apparently, a decision by the authorities not to invoke legal sanctions against those who seek its assistance. (Homosexual acts carry a sentence of up to five years in prison.)

A mass campaign of 'special publications' on AIDS is planned, and there is talk of a telephone 'hotline' information service. The All-Union Ministry of Health is to establish a network of diagnostic laboratories which will ultimately screen the blood of all donors as well as all patients presenting symptoms indicative of AIDS. A massive training scheme for specialists in diagnostic and treatment of AIDS is also planned. A new specialized clinic for AIDS will be established, and the 40-odd institutes working on AIDS have been promised increased funding.

The turnabout is not entirely unexpected. There have been several hints that Soviet doctors and virologists would be interested in cooperating with the West on AIDS research — "in the interests of science". The new AIDS awareness campaign was first disclosed at the end of last year, but the full-scale information campaign seems to have been held up until sufficient test-packs were available to meet the expected demands. Interviewed by the Moscow *Literaturnay Gazeta* last week, the deputy minister of health, Georgii Khlyabich, castigated Western companies that kept their work on AIDS diagnostics so secret that "we obtained practically no information at all, even though we visited those firms". The Westerners, he implied, were dominated by the profit motive. Soviet scientists, he said, have therefore had to seek their own solution. Intensive research on the "diagnostics amd prophylaxis" of AIDS began in 1985, Khlyabich said, and a Soviet diagnostic preparation is now being produced on an industrial scale. Known as IFA-SPID (SPID — pronounced "speed" — is the Russian acronym for AIDS) the preparation, which was developed by a team headed by Dr Viktor Zhdanov and Dr O. G. Andzhaparidze, is said to be "identical in sensitivity and effectiveness" with Western analogues.

At the same time, a team at the Institute of Immunology of the Academy of Medical Sciences headed by Dr Rem Petrov is working on a synthetic diagnostic agent, Khylabich said. According to a TASS interview with Petrov, this agent is based on synthetic peptides and known as Peptodskrin. Petrov's team is trying the same approach to develop a vaccine to AIDS.

Until such vaccine is developed, however, (and Khlyabich puts the time scale "at least five years") the main means of AIDS prevention must be what Dr Valentin Pokrovskii of the Central Institute of Epidemiology called "a sober way of life in all its manifestations". Interviewed on Moscow radio, Pokrovskii said that Soviet "social conditions" insure against a major epidemic in the Soviet Union, and urged the advantages of an ordered sexual life. It is those who have had sexual contact with foreigners, he said, who should report for check-up. Mr Gorbachev's campaign against drug addiction and alcoholism, he said, was a major means of AIDS prevention. Pokrovskii said the risk from blood transfusions is negligible, as the Soviet Union buys neither blood nor blood-products, and tests on a "sufficient cohort of [Soviet] donors" has revealed no infection with AIDS.

But last October, Zhdanov said that AIDS was first discovered in the Soviet Union two years before in an 11-year-old girl who before contracted it during a blood transfusion, and, according to the Khlyabich interview, Zhdanov fears an eventual incidence in the Soviet Union of one in 100,000 of the population (say, 2,800 cases).

But whatever the scale of the eventual spread of the disease, the AIDS campaign is presenting considerable logistical problems for the Soviet health service. The endemic lack of drugs and equipment has been a major target of Gorbachev's drive of "open" criticism, and the extra burden of the AIDS campaign will fall on a medical supply industry already far behind target in both quality and quantity. Nor is it only AIDS-related equipment and drugs that will be needed — the entire Soviet medical profession will switch over to the use of disposable syringes. **Vera Rich**

Investment by Japan booms in Europe and US

London

While the European Economic Community (EEC) prepared its anti-dumping case against the Japanese semiconductor industry this week and the US trade authorities once more accused the Japanese of breaching trade agreements, a British report disclosed an embarrassingly high level of inward investment by the Japanese in Europe and the United States.

The European anti-dumping suit, presented to a meeting of the General Agreement on Tariffs and Trade (GATT) at the beginning of this week, cites Japan as exporting microchips to Europe at below their market value. The United States has made similar accusations in recent months but has not confined criticisms of Japanese trading practices to the semiconductor industry. There is growing concern among the US trade authorities that the Pacific basin industries are the primary cause of the US trade deficit.

The figures, highlighted in a report by the Royal Institute of International Affairs, show that more than a third of Japanese overseas investment in the past 35 years has been directed at the United States, accounting for a commitment of $25,290 million. The figures for Europe are equally stark. In the same period, Japan has poured some $11,000 million into Europe — more than 13 per cent of Japan's overseas investment.

The size of the investment in both continents is likely to support the view that Japan is using the finance from its trading surpluses to erode indigenous manufacturing and service industries, thereby further weakening the competitive challenge of the United States and Europe.

But the figures also indicate that the investment has proved to be a valuable source of new jobs, again a situation with which indigenous industry has expressed its unease.

According to the study, *Industrial Collaboration with Japan*, the United States is "by a long way" the first choice for Japanese investment. By the end of 1984, there were 370 Japanese manufacturing operations in the United States with much investment being attracted by the overseas marketing efforts of individual states, desperate to create new jobs. By last year, claims the study, more than 20 state governments had individual offices in Tokyo.

Not all have been happy with this approach. Colorado governor Richard Lamm is cited in the report as highly critical of the strategy and the dangers posed to US technology. Bill Johnstone

Students in India object to AIDS test

New Delhi

THERE is no indication that the Indian government will reverse its decision on compulsory tests for AIDS (acquired immune deficiency syndrome).

The Minister for Human Resource Development, Mr P.V. Narasimha Rao, told the Indian parliament on 25 February that the screening of foreign students for AIDS would continue. Rao ignored suggestions from Members of Parliament that this discriminatory practice could spoil India's relations with African countries. He said AIDS is a serious matter and that the government has decided to deport foreign students who carry the AIDS virus. He also rejected a suggestion that serum samples suspected to be positive for AIDS, on the basis of tests carried out in India, should be sent abroad for confirmation.

Africans, who constitute more than 80 per cent of the estimated 30,000 foreign students, feel the test is directed at them and say it is racist. The foreign student community does not oppose tests on new entrants but is against screening those who have studied in India for several years.

Foreign students of Bombay University failed to turn up for the test scheduled for 16 February. The screening was postponed to 22 February but the International Students Association decided that none of its members would take the test unless local students were also included. Foreign students at Delhi University have also boycotted the test.

Fear, social ostracism and the psychological trauma of being singled out as prospective AIDS carriers have put the educational career of the Africans in jeopardy, their student leaders allege. They say they are shunned by their former Indian friends in the universities, in their apartments and by tradesmen. The Africans fear expulsion from the universities on the basis of unreliable tests showing false positives, or tests conducted by non-experts. They claim that three Kenyan students deported recently from India were found to be AIDS-free in more accurate tests carried out by experts back in Kenya.

The students are agitated because they have been told that failure to take the test would prevent them taking the annual examinations, barely a month away.

Some 1,500 African students who have refused to undergo the test were planning a protest march in New Delhi on 27 February, and it was expected that a large number of sympathizers would attend.

India has so far detected 92 cases of AIDS infection, five of whom died in the past year. K.S. Jayaraman

Extra MRC funds earmarked for AIDS research in Britain

London

BRITISH scientists have persuaded the government to increase the budget of the Medical Research Council (MRC) by £14.5 million over three years for research into the prevention and cure of AIDS (acquired immune deficiency syndrome). Most unusually for the MRC, the research is to be centrally directed in an effort to maximize its efficiency.

There will be two programmes of research, one on vaccines and the other on drugs. Each will be organized by a separate director who has yet to be appointed. More of the money is expected to be spent on vaccine research than on drug research, says the MRC's secretary Sir James Gowans, not least because the drug research is more likely to attract industrial sponsorship. £2.5 million will be available for both programmes this year, with £5 million next year and £7.5 million in 1989. Gowans says he has received assurances of longer-term funding.

The two directors will be responsible for commissioning the various strands of their programmes of research. Much of the research will probably be concentrated in three or four large centres with smaller, specific projects being commissioned elsewhere. Both Gowans and Mr Norman Fowler, Secretary of State for Social Services, emphasized that contracts will go to the most appropriate laboratories, whether they are academic or industrial.

The proposals that attracted these funds were put together by the MRC after consultations with scientists, many of whom would be keen to play a part in the project. Gowans was closely involved in the consultations on vaccine research, while Sir David Phillips, chairman of the Advisory Board for the Research Councils and a leading protein crystallographer, chaired a recent and hurried meeting of molecular biologists with a potential interest in exploring viral proteins that could be targets for drugs against AIDS. Both sides agreed that the two major limiting factors in AIDS research are the lack of sufficient quantities of viral proteins either to be tested as vaccines or to be crystallized for structural studies and the lack of convenient animal models.

Speaking at MRC's press briefing, Professor William Jarrett of the University of Glasgow, said that some of the funds earmarked for vaccines will have to be spent on animal testing facilities. Chimpanzees and, perhaps, gibbons are the only animals that can currently serve as models but the recent report of an HIV-type of virus associated with an AIDS-like syndrome in cats was hopeful. He believed there would soon be immunity trials of subunit vaccines in man. Gowans volunteered himself for such a trial.

At least two laboratories have expressed an interest in filling the need for large quantities of viral proteins. One is the Public Health Laboratory Service Centre for Applied Microbiology and Research, at Porton, which already produces viral antigen for Wellcome's AIDS test. The centre's director, Dr Peter Sutton, has already discussed with MRC how the centre might help. The other is the Natural Environment Research Council's Institute of Virology in Oxford which has expertise in the use of engineered baculoviruses for the production of large quantities of proteins. Its director, Professor David Bishop, is waiting to hear whether the MRC is interested in this project. If not, he has commercial backers in the wings.

Research on the proteins, once available, is likely to proceed in various laboratories. Dr John Skehel's division of virology at the MRC's National Institute of Medical Research is already working on the envelope glycoprotein in conjunction with crystallographer Don Wiley at Harvard. And Professor Tom Blundell's group at Birkbeck college in London is particularly interested in the proteinase of the virus, which has some relationship to proteinases of known structure. One centre for vaccine research seems certain to be Jarrett's laboratory. **Peter Newmark**

Compulsory AIDS testing in Bavaria

Hamburg

ANYBODY suspected of being infected by human immunodeficiency virus (HIV), the cause of AIDS (acquired immune deficiency syndrome), will soon be compelled by the Bavarian authorities to undergo a test.

The decision was made by the cabinet (*Ministerrat*) of the Bavarian *Land* government on 25 February. If such people do not appear for testing the health authorities and police will force them to do so.

Male and female prostitutes fall into the risk category, as does anybody seen talking to them. Prisoners and those held on remand are also compelled to take the test. Furthermore, candidates for the civil service will be tested, and anybody whose test proves positive will be rejected.

The other states of the federal republic have criticized the tough stance of Bavaria. The federal minister of health, Rita Süssmutt (Christian Democrat), said that this was the wrong way to deal with the disease. The Social Democrats called the Bavarian initiative "ineffective and hardly acceptable". Claus P. Dechau

AIDS meeting calls for wider testing

Washington

TESTING for AIDS (acquired immune deficiency syndrome) should be offered more aggressively to people in high-risk groups but should not be made compulsory. That is the conclusion of a conference sponsored by the Centers for Disease Control (CDC) in Atlanta last week.

The participants, including state and local public health officials and civil liberties advocates as well as representatives of some high-risk groups, met to discuss the role of serological testing in the prevention and control of AIDS. The CDC will evaluate the recommendations and issue its own report to the government in the next few weeks.

The biggest change that the conference participants advocated was a shift from passive to active testing in treatment centres for sexually transmitted diseases and intravenous drug users. Under this plan, people attending the centres would be tested for AIDS "routinely" unless they specifically objected.

The participants also called for greater confidentiality of test results and protection from discrimination of those tested. Although unauthorized release of test results does not seem to have occurred very frequently, some groups, particularly homosexuals, fear the repercussions of unauthorized release.

Nevertheless Franklyn N. Judson, director of public health in Denver and co-moderator of a panel discussion at Atlanta, stressed the need for keeping confidential records of AIDS virus carriers. He pointed out that for years there have been confidential registries for people with other sexually transmitted diseases, and that it "would be irresponsible not to do this for AIDS". But Judson thinks it unlikely that the US government will issue regulations on confidentiality.

Judson said that cities such as Denver have begun screening blood from hetero sexuals who attend the treatment centres for sexually transmitted diseases. So far, all carriers of the AIDS virus have come from an identified high-risk group.

CDC are also conducting a long-term study of blood scheduled to be discarded from hospitals for evidence of AIDS virus antibodies in the hope of compiling data on the prevalence of infection. All patient identification data except for age and sex are removed before blood samples are tested. The hospitals involved are in both low- and high-prevalence areas. By using blood already due to be discarded, the CDC are able to avoid issues of consent.

Steven Dickman

Special Research Centres to continue in Australia

Sydney

THE Australian government is to continue to fund the Special Research Centres says Senator Susan Ryan, Minister for Education. The centres were previously known as 'Centres of Excellence' at their inception in 1982.

There are at present nine Special Research Centres, which last year received a total of A$6 million. On the recommendation of a Commonwealth Tertiary Education Commission (CTEC) committee, Ryan has promised that funding for the programme will continue at least at the present level for the three years to 1990. One and a half centres will lose their status as such this year and the rest will suffer cuts of around 10 per cent. The A$1.5 million saved will go to establish three new centres that will be announced in the federal budget in August. The new centres are likely to be in areas closely related to industry to increase exports of manufactured goods and to answer criticisms that the present distribution is biased towards medicine and biology.

To be cut off this year are the Centre of Policy Studies at Monash University headed by Professor Michael Porter and the group led by Professor R. Bruce Knox working on reproductive biology, which makes up half of the Plant Cell Biology Research Centre at the University of Melbourne, the surviving half being led by Dr Adrienne Clark, the only woman leader among the centres. The axing of Porter's centre was seen by the Conservative opposition as a blatant political move by the Labor government as the centre has been involved in modelling work for the development of tax policies for the opposition and right-wing groups. Ryan maintains that the move was taken purely on the advice of the CTEC committee which concluded that the published work attributed to the centre of neither the quantity nor the standard required.

The Special Research Centres are opposed by the Federation of Australian University Staff Associations (FAUSA) which considers the centres' budget to be taken from that available for research by the average teaching academic.

The centres were established by the previous Conservative coalition government on the premise that applying concentrated money with guaranteed continuity to the nation's best brains would produce results far in excess of what might be achieved if the money were spent through normal channels. Dr James Beattie, associate professor in chemistry at Sydney University and chairman of the FAUSA Research Committee, points out that the CTEC review was conducted hurriedly with some the centre assessment panels complaining of lack of time.

According to Beattie, FAUSA would prefer to see the money spent on the general research infrastructure such as buildings, libraries and equipment, at present in a poor state. FAUSA would prefer the funds to be administered by the Australian Research Grants Scheme (ARGS) where the relative excellence of proposed research is subject to broad-based review by academic peers.

Each year, the ARGS distributes about 1,200 grants to a total Australian academic population of about 12,000 scientists. Alternatively, given that the average ARGS grant is worth A$20,000, the A$6 million of special research centre money would be enough to fund the work of the 300 researchers whose proposals the ARGS judged to be "excellent" but for which it had no money in 1986.

Charles Morgan

Boost for Indian high-energy physics

New Delhi

INDIAN physicists are making plans to build a 1-GeV proton synchrotron, to be followed by a 12-GeV Synchrotron, in an attempt to catch up with the West in high-energy physics research. The accelerators will be built at Indore, in Madhya Pradesh, where India is establishing the Centre for Advanced Technology (CAT) at a cost of some $100 million.

The task of setting up CAT has been given to the Department of Atomic Energy (DAE), which says that the centre will focus on accelerators, lasers and laser technology for thermonuclear fusion. CAT is expected to become a major high-technology centre in the next 20 years.

To start with, DAE hopes to build a synchrotron radiation source consisting of a 25-MeV electron linear accelerator, a booster that will accelerate the electrons to 700 MeV, and two storage rings. The proton synchrotrons are aimed at producing anti-protons and kaons and studying the physics of condensed matter using heavy ions. India at present has a 100-MeV cyclotron in Calcutta, a 5.5-MeV Van der Graaf machine and a 14-MeV pelletron in Bombay.

According to DAE, CAT will be the main research centre for work on laser fusion. CAT will also concentrate on associated fields such as cryogenics and radio frequency systems.

K.S. Jayaraman

How to lubricate the gears of Texas higher education

Austin

ALTHOUGH the major centres of higher education in the United States are still on the country's two coasts, Texas universities have been making steady progress towards national prominence. When oil flowed freely, universities in Texas enjoyed the boom. Now, with the state's oil-based economy depressed, higher education is struggling.

When a special session of the legislature last year had to cut $512 million from the state's $19,000 million budget, $200 million came from funds for higher education. But supporters of higher education are preparing to fight as the next round of the budget debate gets under way to restore funds, allowing Texan universities to continue their improvement.

Ammunition for the fight comes from a report released last week by the select committee on higher education. Recognizing that the state's financial difficulties threaten earlier gains in higher education, the state legislature in 1985 created the select committee to consider the problem. After 15 months, including some 30 all-day sessions, the committee's recommendations encompass a variety of issues ranging from faculty salaries to requirements for undergraduate degrees and state support for basic research at universities.

One recommendation that appears to have strong backing is state support for university research. In 1985, the legislature created the Texas Advanced Technology Research Program(TATRP), providing $35 million for competitive research grants for scientists in the state's public universities. Grants were awarded to five areas, based on a National Academy of Sciences report which identified promising research areas.

Norman Hackerman, formerly president of the University of Texas at Austin and Rice University and a member of the select committee, says TATRP gave the government of Texas a chance to "get its feet wet" in research support based on peer review. Hackerman headed the committee's task force on research, which has recommended continuing and expanding the TATRP programme. As well as supporting technology, the report urges the state to award between $20 and $40 million — approximately 10 per cent of the amount from the federal government — for basic research at universities. The report also recommends that institutions should receive half of the indirect cost reimbursement accompanying federal grants.

William Hobby, champion of higher education.

But peer-reviewed distribution of funds presents political problems. The panel chosen to divide TATRP funds would have given 90 per cent of the money to researchers from the University of Texas at Austin and Texas A&M University at College Station. But legislators, jealous of their already high status, prevented them from receiving more than two-thirds of the TATRP funds.

Admiral Bobby Inman, formerly president of the Texas-based Microelectronics and Computer Technology Corporation and originator of the TATRP concept, believes a form of intellectual elitism is ultimately good for the state. In hard economic times, it makes good sense to put resources where they will do the most good, says Inman. If that means just a handful of institutions win grants, so be it.

Resolving regional differences occupied a large portion of the select committee's energy. Economic necessity seemed to require closing some institutions opened during more prosperous times. But closing a university is "harder than moving a cemetery", says Texas commissioner of higher education Kenneth Ashworth. Many communities appeared before the board to plead their local university's case. One school even rented a hot-air balloon to fly over Austin to protest at its potential demise. In the end, the committee opted to recommend retaining all 37 universities, 49 community colleges, 4 technical institutes and 8 medical and health-related institutions. Instead of closing universities, the committee recommends that some institutions abandon their graduate programmes and stick to undergraduate education. But even that will be opposed.

Ashworth says this is a time for higher education to put the brakes on expansion, and to find ways to guarantee support for existing institutions. He is the first to point out that the cuts in the Texas education budget have had a serious and detrimental impact on faculty recruiting. Texas faculty members earned 10·2 per cent less than the average paid to faculty members in the ten largest states a year ago (Texas is third largest). That figure is expected to reach 14·4 per cent in the current academic year. Unless the new state budget provides more salary funds, Texas universities will have tremendous problems recruiting and retaining staff, says Ashworth.

Hans Mark, chancellor of the University of Texas system, agrees with Ashworth that raising salaries is critically important to maintain the health of higher education. But he "emphatically disagrees" with Ashworth's suggestion that the time is not right for expansion. Mark is actively seeking both federal and private funds to help establish new facilities and attract top talent. Mark believes there is reason for optimism about state revenues. He suggests that if oil prices reach $20 per barrel, and certain short-term increases in sales tax are extended, possible budget shortfalls will not materialize.

A powerful champion of higher education in Texas is Lieutenant Governor William Hobby. A member of the select committee, Hobby has been lieutenant governor since 1972 and is well versed in the intricacies of Texas politics. Hobby talks with pride of the state's academic achievements but bemoans the fact that Texas has less than one-tenth the number of members of the National Academy of Science that California has. Hobby has worked hard to persuade the state's congress that a strong educational system is needed to restore Texas to economic health.

But there is a danger that selling education for its economic benefits may backfire if the economy does not improve. Larry Temple, an Austin lawyer who chaired the select committee, says it is hard to get politicians to take a 20 or 30 year perspective on the benefits of education. But he likes to remind people that, no matter what size the Texan oil reserves may be, it will take educated minds to find them.

Joseph Palca

New Wellcome unit

London

TAKING £1 million from its swollen coffers, the Wellcome Trust has set up a new unit within the University of Glasgow. The Wellcome Unit of Molecular Parasitology, directed by Dr Andrew Tait, is to concentrate on molecular and genetic investigations of the basic biology of parasites.

The unit, which is guaranteed for five years, is the first to be funded by Wellcome Trust since it announced that it would be looking for suitable long-term university projects to fund with the extra income derived from its sale of 25 per cent of its (until then wholly-owned) shares in Wellcome plc, the pharmaceutical group whose share have doubled in value in the last month largely because of the anticipated sales of Retrovir (AZT) as a treatment for AIDS.

Peter Newmark

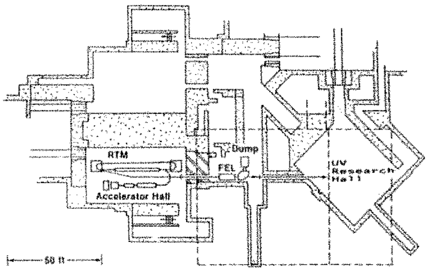
SDI gives NBS a free-electron laser for basic and applied research

Washington

RESEARCHERS using high-intensity radiation sources will soon have an important new research tool. The National Bureau of Standards (NBS) in building a Free-Electron Laser (FEL), due to be completed in 1990, at its laboratories in Gaithersburg, Maryland. Unlike other FELs constructed so far, this will operate at wavelengths in the near-ultraviolet and visible regions as well as in the infrared, and at a much higher average power. It will be used in fields ranging from atomic physics to nuclear medicine.

The $4.9 million cost of the FEL will be borne by the Strategic Defense Initiative Organization through a contract administered by the Office of Naval Research and the Air Force Office of Scientific Research. The FEL will give its designers familiarity with the operation of free electron lasers. This knowledge could then be applied to the construction of lasers operating at even shorter wavelengths, perhaps for military purposes.

The new laser was conceived by FEL theorists at the Naval Research Laboratory as an adjunct to the 185 MeV continuous-wave 'racetrack' electron accelerator already under construction at NBS. The accelerator acts as an energy source for the laser by providing regular pulses of electrons which, by interaction with carefully shaped irregular magnetic fields, give off energy as radiation.



Plans of the free-electron laser and racetrack microtron.

According to NBS physicist Xavier Muruyama, people are "throwing away a lot of available light" with the lasers now in use. The continuous-wave accelerator, or 'microtron', will allow the new FEL to operate over a much broader spectrum of wavelengths than other free-electron lasers. Its range will extend from the 10 μm infrared region to the ultraviolet region at 0.2 μm. Furthermore, because the repetition rate of the electron pulses feeding the laser will be essentially the 100 MHz frequency of the microtron, the average power output of the FEL will reach hundreds or even thousands of watts.

The remarkable range of wavelengths is due both to the microtron's power and to its design. Electrons can be deflected at several points along their path through the accelerator. The full 185 MeV of energy, corresponding to the shortest wavelength, will be achieved only when electrons pass through the entire microtron. Adjustments to the mirrors in the laser itself will be required to provide this broad range, but project director Samuel Penner considers this problem easily surmountable.

The fact that the FEL will be tunable over such a broad range of wavelengths may make it particularly suitable for medical research. For example, researchers at Harvard Medical School are using lasers in experiments on animals to break up cholesterol deposits in arteries. These experiments depend on fine-tuning the wavelength so that the laser beam destroys the blockage without hurting the artery wall. (The FEL itself will not be useful for surgery until fibre optics are developed that can carry enough power.) In addition, the Food and Drug Administration is interested in using the FEL to test the efficacy and safety of the lasers now used in medicine.

Penner says that the fact that light pulses from the FEL will be synchronized with the pulses of synchrotron radiation from the microtron means that the FEL will be especially useful for 'pump-probe' experiments in which a pulse of light is used to excite an atom or molecule to a higher energy, and a second pulse to analyse the effects of the first. The high power provided by the laser will allow the FEL to 'read' these excitations even if it is not tuned to exactly the right wavelength. Acording to Penner, the NBS laser will be the first FEL to be used for such a variety of tasks.

Steven Dickman

Planetary scientists appeal for 1990 launch of Mars Observer

Washington

THE US Planetary Society, headed by chief spokesman and standard-bearer Carl Sagan, rallied support on Capitol Hill two weeks ago for the Mars Observer, a NASA (National Aeronautics and Space Administration) planetary mission left temporarily grounded in the aftermath of the Challenger accident. In evidence to the Senate Subcommittee on Science, Technology, and Space, Sagan stressed the importance of the Mars Observer as a step towards (ultimately manned) exploration of Mars, an objective he suggested would revitalize sagging NASA morale.

The Mars Observer, now set for a 1992 launch, was originally to leave Earth in 1990 to spend a martian year (roughly 687 Earth days) in a low Sun-synchronous orbit monitoring the magnetic fields, atmospheric circulation, gravity fields and chemical composition of Mars by remote sensing. It would also gather data on seasonal fluctuations in the parameters determining geochemical cycles and would provide clues to what happened in the planet's climatological history to dry up the once-extensive water supplies.

But the shuttle timetable until 1990 has only three slots for planetary missions, now filled by the Galileo mission to Jupiter, the Magellan radar mapper mission to Venus and the Ulysses mission to explore the polar regions of the Sun. Ulysses, a joint project with the European Space Agency (ESA), has already been delayed several times. Further delay could be acutely embarrassing to NASA.

The decision to postone the Mars Obsever launch until 1992 provoked and outcry from the planetary science community and a letter-writing campaign, sponsored by the Planetary Society, which resulted in over 20,000 letters to congressmen and NASA officials.

Delaying the launch may put the United States behind the Soviet Union in the exploration of Mars. The Soviets have recently brought forward from 1994 to 1992 plans to deploy a balloon probe to the surface of Mars, coinciding with the proposed launch date for Mars Observer. Next year, the Soviet Union will also launch a remote sensing mission ot the Martian moon Phobos; former NASA Jet Propulsion Laboratory director Bruce Murray says their plan is "extremely elegant".

Sagan, Murray and Congressman George E. Brown Jr. (Democrat, California) advocate a joint programme of Mars exploration with the Soviet Union. Sagan says a national commitment to put a man on Mars would have the same effect as the Apollo lunar programme, providing a unifying goal to put NASA back on track.

The shuttle is still due to launch Mars Observer, although expendable launch vehicles, such as the Air Force Titan 34D, are acceptable alternatives. But Congressman Edward P. Boland (Democrat, Massachusetts), chairman of the House of Representatives appropriations subcommittee that oversees NASA, objects to the Mars Observer using an expendable rocket. He claims the extra $100 million needed to launch Mars Observer in 1990 using a relatively outdated Titan 34D is a poor investment, considering the impending development of a more advanced Titan IV rocket schedules for 1991. But waiting until 1992 will add an additional $90–100 million to the price.

Carol Ezzell

Japanese Frontiers programme fillip to research

Japan

THE Human Frontier Science Program, Japan's answer to Star Wars and Eureka, could be the best thing that has ever happened to bioscience research in Japan. Much will depend on the next few weeks and months as the ministries involved debate the recommendations of foreign and Japanese scientists and try to secure financial support from the Ministry of Finance, Japanese industry and foreign governments.

The Human Frontier Program, as it used to be called, first emerged from the Ministry of International Trade and Industry (MITI) about a year ago (see *Nature* **320**, 296; 1986) alongside a similar proposal, the Human and Earth Science Program, put forward by the Science and Technology Agency (STA). Both advocated international research in basic bioscience for the 'benefit of all mankind' and agreed with the Maekawa report, released shortly after, which recommended that Japan should contribute to new science and technology in the 21st century by promoting international collaboration in basic research.

Frontiers is aimed at defusing Western criticism that Japan is only a consumer of the fruits of basic research. There was a hint that the Prime Minister, Mr Yasuhiro Nakasone, would propose the programme at the Tokyo summit in May last year. But competition between the STA and MITI, the vagueness of the Frontiers programme, and a busy summit schedule led to postponement.

Nakasone, who has long been a backer of the life sciences (see *Nature* **240**, 185; 1972), continues to push the programme. Last year, after behind-the-scenes negotiations between seven ministries, Frontiers re-emerged as the Human Frontier Science Program, a compromise, at least in title, between the MITI and STA proposals.

In mid-December, a feasibility study committee and two working groups of top Japanese scientists were created to develop the programme under the leadership of Michio Okamoto, former president of Kyoto University and chairman of the Committee on Policy Matters of the Council for Science and Technology (headed by the prime minister). Several meetings of both the committee and working groups have been held, including two on 29 January and 12 February at which foreign scientists from the summit nations and the European Economic Community were invited to give their views on how the Japanese government programme should be run.

A 94-page report compiled by MITI's Agency of Industrial Science and Technology in November last year formed the basis of discussion. This report proposes the creation of all the necessary components of an android (artificial brain, eyes, organs, muscles and tissue) through the study of functions of the human body. These 'genuine intelligent robots' might help solve the 'software crisis', Japan's inability to produce good software, and help understand 'technostress', an ailment brought on by working at the man–machine interface.

Most of the report's proposals are less ambitious although on a large scale. The first chapter covers the study of biological functions at the molecular and cell level.

The second chapter deals with functions of the whole organism such as hearing, sight, smell, touch, motor function and thinking and the third with the technology to carry out the research, including the development of an X-ray microscope, a 30T NMR, and ion- and electron beam-probes.

But the report represents only MITI's views. Okamoto and several other scientific advisers favour a programme that would place more emphasis on pure research. In contrast, MITI believes that research should have commercial applications and that pure research will not win funding from the Finance Ministry. The problem is deep-rooted. Engineering has dominated Japan's university system since its establishment in the last century, pure science taking a peripheral role, and, for most Japanese, research means applied research.

Among other possible projects now under consideration is sequencing of the whole genome, one of the original proposals for the STA's Human and Earth Science Program. The STA, in collaboration with Seiko, has been developing an automatic DNA sequence analyser, and the leader of this project, who chairs one of the Frontiers working groups, has long advocated automated processing of DNA sequences such as the human genome. Genome sequencing would fit in with the proposed 20-year timescale of Frontiers and there have already been calls from the United States for collaboration with Japan and Europe on this huge project. But there are fears that megasequencing would absorb too large a proportion of the Frontiers budget. MITI is hoping for Y40,000 million ($270 million) a year (although the budget may be less in the beginning). Most of this would have to come from the government, but industry is expected to contribute. Nakasone will almost certainly propose the Frontiers programme at the next summit in Venice in June, and Katsuhiko Umehara, deputy director of MITI's feasibility study, believes that some other countries will contribute funds.

Sir Walter Bodmer, director of research at the Imperial Cancer Research Fund, said at the meeting on 12 February that he thinks "it is most unlikely that within the United Kingdom we would find a large amount of extra money, if any extra money, to support such a program". But there is "enormous scope for collaboration" within the framework of existing activities and institutions, "if the programme is of a high quality and attractive". Other overseas participants concurred.

Both Bodmer and Professor Joshua Lederberg, president of Rockefeller University, urged Japan to include research directed at the understanding of human diseases, such as parasitic, viral and genetic diseases.

According to a tentative plan outlined at the 12 February meeting, Frontiers will take the form of a granting agency (like the Rockefeller Foundation) which will distribute research grants to international research teams; researchers and information will be exchanged through an international fellowship programme for young researchers and international workshops (like the Gordon Conference) and domestic (Japanese) basic research would be considerably enriched and reinforced in line with the programme.

If the programme is implemented and Frontiers supports basic research, it will be a major turnaround in Japanese science policy. Until now, the Finance Ministry has been frugal on funding basic research. But with the backing of two powerful ministries (MITI and STA) and the prime minister, funding of the magnitude envisaged may well be possible.

One hundred and fifty million yen ($1 million) has been allotted to the feasibility study in fiscal year 1987 on top of the Y20 million for this fiscal year, a clear indication that the government is committed to Frontiers. And Japan will invite ten eminent scientists from around the world to discuss the programme in a "wise men's conference" at the Royal Society in London on 1 April.

The other five ministries represented at the Frontiers meetings (the Foreign Ministry, the Ministry of Education, Science and Culture, the Health and Welfare Ministry, the Ministry of Agriculture, Forestry and Fisheries and the Ministry of Post and Telecommunications) officially maintain a neutral stance on the programme. But the successful launching of Frontiers at the summit would be in the interests of the Foreign Ministry. The Education Ministry, on the other hand, is worried that Frontiers could cut into its science budget, but that would be counter to the spirit of the programme and the Maekawa report's recommendation to promote basic research. **David Swinbanks**

Commercialization of British radio given an amber light

London

UP to three national commercial radio networks and hundreds of community radio stations will emerge from the UK government's tacit approval dramatically to change the rules that constrain the number of UK radio stations.

The new guidelines, published in the form of a green paper (consultation document) last week entitled *Radio: Choices and Opportunities* (Cmnd 92, HMSO, £5.00), will end the monopoly of local commercial stations. Most of these stations enjoy sole commercial broadcasting rights in their areas and may now be challenged by community radio and national networks.

New broadcasting legislation will need to be introduced in the next session of parliament to allow the change. The British Broadcasting Corporation (BBC) will lose two of its seven frequencies. It will be given one in return in 1990 when more frequencies become available. International agreements will provide additional VHF/FM frequencies for broadcasting between the end of the decade and 1995. The government says these will provide "scope for new national and local services".

The national commercial radio service could fuel controversy. The advertising revenue on which the local stations depend could be threatened.

But the national network may have a higher proportion of speech to music and should be more of a challenger to the BBC's news channel, Radio 4, than the local commercial stations. That at least is the idea of the Independent Broadcasting Authority (IBA) currently the watchdog of the commercial radio stations.

Douglas Hurd — planning a radio revolution

The government does not want to restrict any commercial venture and is unlikely to impose a formula like that suggested by the IBA. At the community level there is expected to be an explosion.

Mr. Douglas Hurd, the Home Secretary says that "The values and traditions established by our history of high-quality public service broadcasting should not be lost, there can soon be a more varied pattern of radio services and more choice for the listener".

Bill Johnstone

US Congress debating aid for Pakistan

Washington

SPECULATION that Pakistan has "crossed the nuclear weapons threshold" put a crimp in last week's congressional hearings about a $4,020 million US aid package to Pakistan. The Reagan administration wants the six-year package, an increase of $800 million over the last allotment, for economic and military support of Pakistan in the face of the continued Soviet presence in Afghanistan. But Congress is expected to ask some tough questions about Pakistan's nuclear capability.

Since 1985, US aid has been contingent on the US president verifying to Congress each October that Pakistan does not have a nuclear device. A report released this week by the Carnegie Endowment for International Peace indicates that Pakistan either "possesses all of the components needed" to manufacture an atom bomb or else "remains just short of this goal" because of a lack of enriched uranium.

The report, written by Leonard Spector, recounts the history of Pakistani attempts to acquire nuclear weapons technology. Spector cites as one example the purchase of a plutonium reprocessing facility from France in the mid-1970s which would have permitted Pakistan to accumulate nuclear weapons material. The United States cut off aid to Pakistan in 1976 over precisely this issue. Deliveries for the facility were ultimately suspended and US aid reinstated in 1977. Aid was cut off again in 1979, when it had been found that Pakistan had been acquiring technology for uranium enrichment, and was restored only in 1981, after Soviet forces appeared in Afghanistan.

The administration recently warned Pakistan that US aid may be cut off once again if Pakistan is found to possess even the makings of a bomb. In a speech in Islamabad on 16 February, US Ambassador Deane Hinton warned that under such conditions, it would be "open to question" whether the president could make the necessary certification. Pakistan continues to deny that it is developing nuclear weapons. Congress will decide on aid in the next few months.

Steven Dickman

Government and students make peace in Spain

Barcelona

THE Spanish minister of education and science, José Maria Maravall, has signed agreements with most of the student organizations that led demonstrations against the Spanish educational system during the past two months. Some radical student organizations have decided to continue the movement, but most students have returned to their classrooms. The agreement is based on a proposal by the minister that includes a gratuity for secondary school students under 16 and for university students from families under a certain income, a substantial increase in the number of fellowships, reforms in the secondary schools and a revision of the entry requirements for universities.

Both sides claim victory. The students say they have forced the minister to negotiate and to accept some of their points. He in turn has declared that the agreement does not mean a departure from his policy but only an acceleration of the reforms already under way.

Most students have returned to class but the unrest has spread to previously unaffected universities. At the Autonomous University of Barcelona police entered the campus to expel students occupying the main administrative building, and the university was closed for two days. This was the first such incident in Spain for more than ten years.

It is clear that the agreement between the minister of education and students will be costly. It is calculated at about 40,000 million pesetas per year (£200 million), almost twice the budget of the CSIC (the Spanish research council). It is not clear where the money will come from and some people fear that proposed increases in university or research funds or salaries may be frozen as a consequence.

Pedro Puidoménech

Evolution and optimization

SIR—Joe Felsenstein opens his review[1] of Sewall Wright's biography by W.B. Provine[2] in these words: "Evolutionary theory in the 1960s and 1970s was dominated by optimization, by the search for the quantity evolution would maximize. The mean fitness of a population was not precisely what natural selection would maximize in a Mendelian genetic system, so perhaps if we did enough theory we could find out what was. The picture hanging on our wall was of R.A. Fisher, who had emphasized the importance of natural selection in large populations."

This might be taken to imply that Fisher engaged in the search to which Felsenstein refers, as many seem to think. This is not the case; indeed, his students from the late 1950s (I write as one of them) were taught *not* to think in such terms, and we developed a marked scepticism towards those many who did. As early as 1941, Fisher[3] railed against any such optimization: "Wright's conception ... that selective intensities are derivable, like forces in a conservative system, from a simple potential function dependent on the gene ratios of the species as a whole, has led him to extensive but untenable speculation."

To this evidence can now be added the recently published letter[4] that Fisher wrote to M. Kimura on 3 May 1956: "In considering the original statement of what I ventured to call 'the fundamental theorem of natural selection', I had, of course, considered the relation between such a situation and that in which a potential function existed, for my mathematical education lay in the field of mathematical physics.

As you realize, I preferred to develop the theory without this assumption, ... Of course I realise that Sewall Wright has often argued as though such a potential function must exist, ..."

In the matter of optimization, the picture on the wall was that of Sewall Wright, and it carried the caption "The inventor of the notion of the *adaptive topography*, the *adaptive surface*, and the *fitness function*".

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Foreigners in Japan

SIR—As a British scientist temporarily in Japan participating in a programme of collaborative research sponsored by the Japanese Foundation for the Promotion of Cancer Research, I should like to add a comment to David Swinbanks' article "British are reluctant travellers" (*Nature* **323**, 571; 1986).

It is not difficult to understand, particularly when viewing the situation from the United Kingdom, the apprehension about living and working in Japan that may be in the minds of British scientists. However, once in Japan many of the difficulties one expected to face are, in reality, much less serious than they appear from the United Kingdom. In fact, many of the so-called 'problems' are a source of real pleasure in the interest and the very challenge which they present.

The 'challenge' of Japan should be viewed in a positive way because there are so many things to be gained by spending time here. First, it should be recognized that many of the research centres and university departments in Japan are of a very high standard and have truly earned their international reputation for excellence. One can therefore realistically expect the time spent here to be scientifically productive and successful. Of course, one is expected to work hard and the normal working hours are longer than those in the United Kingdom, but this should not be a detraction. In most research groups this is hardly noticeable as there is a strong feeling of companionship and very much a community spirit.

Second, although one might be apprehensive about the language problem and differences in culture, the Japanese are a very generous and helpful people. My colleagues here go out of their way to ensure that, as far as possible, my every need is met, and that the communication problem both inside and outside the laboratory does not lower the quality of my life here.

The concern about finding employment back home is understandable but should not be allowed to over-influence a decision to come to Japan. At a time when efforts are being made by both industry and government to increase British interactions with Japan, experience in Japanese science and Japanese culture must be an advantage. For those who wish to return to an industrial position, I suggest that they make contact with companies before departing for Japan. Certainly, the one-year fellowships that the Royal Society plans to offer to those returning to the United Kingdom should cushion the problem of finding an immediate position.

For those who are offered the opportunity of participating in research in Japan, to fail to accept it is to miss the chance of a lifetime.

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Science in Iberia

SIR—Your report on tropical research in Portugal states that "in the first major outbreak [of coffee leaf rust], towards the end of the nineteenth century, Ceylon's coffee industry was utterly destroyed" (*Nature* **324**, 331; 1986). To be precise, British entrepreneurs began to develop coffee production in Ceylon in 1803, increasing annual production to over one million pounds within 50 years. But the first major outbreak of coffee leaf rust caused by *Hemileia vastatrix* occurred in 1869, and not "towards the end of the nineteenth century"[1]. As it was one of the classic cases in the history of phytogeography of destruction of plantation monoculture by a pathogen, the record should be put straight. Ceylon has been a tea producer since the 1870s.

In your coverage of science in Spain, you record the activities of two Spanish Nobel laureates, Ramon y Cajal (1906) and Severo Ochoa (1959). It would be fair also to mention Portugal's 1949 Nobel laureate in sciences, Antonio Egas Moniz (1874–1955) who is recognized for his pioneering work in prefrontal lobotomy and in cerebral angiography; a junior contemporary of Ramon y Cajal, the Portuguese neurosurgeon was also politically active. He served as a deputy in the Portuguese parliament from 1903 to 1917, when he was named as ambassador to Spain and was also appointed as the Minister of Foreign Affairs. Moniz also served as the president of the Portuguese delegation at the 1918 Paris Peace Conference, before retiring from politics in the following year[2].

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Iberia reversed

SIR—Perhaps it is labouring the obvious, but it seems that the cover of *Nature* **324**, No. 6095, which depicts a Catalonian view of Iberia in 1375, was placed upside down if one subscribes to the convention that north is up. Inversion of the cover might allow more readers to appreciate correlations with modern Iberia.

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Many early maps were turned round in order to make the best use of the paper without altering the format of the completed atlas. — Editor, *Nature*.

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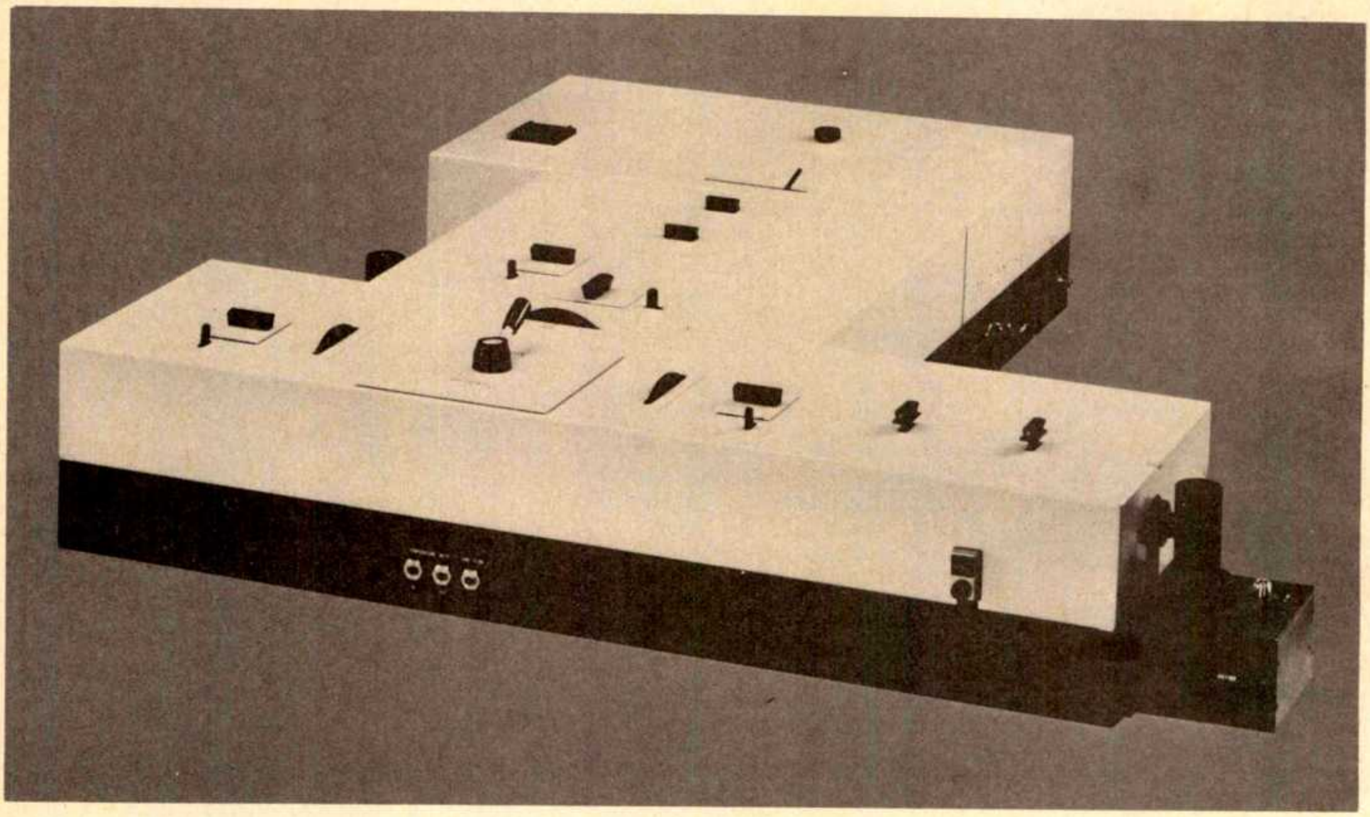

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Supernova almost on the doorstep

Last week's discovery of a supernova outburst in the Larger Magellanic Cloud is an important opportunity for astronomers of several kinds, but one chance has been missed already.

ASTRONOMERS have been handed a potential treasure trove with the discovery last week of a supernova 155,000 light years away in the nearby satellite galaxy, the Larger Magellanic Cloud (LMC). The exploding star, 10,000 times brighter than any other object in the LMC, provides not only an unprecedented opportunity to study the development of a supernova, but also a beacon by which interesting constituents of the intervening matter can be studied. Perhaps most significantly of all, neutrinos emitted by the initial explosion are thought to have been detected. Not surprisingly, every observatory within sight of the object (predominantly in the Southern Hemisphere) and every appropriate satellite has been rescheduled to monitor the event.

Three astronomers share the credit for the discovery on 24 February. Ian Shelton, of the University of Toronto, found the very bright object by good luck on a three-hour exposure taken at the Las Campanas Observatory in Chile. Oscar Duhalde, a night assistant at the same observatory, spotted the source with the naked eye, as did Albert Jones, an amateur observer of variable stars living in New Zealand.

It turns out that this patch of sky had been photographed by other observers over preceding nights, from which it seems that the supernova was first readily observable (at magnitude 6) on 23 February. Since then, it has brightened by about two magnitudes and is expected to reach its peak brightness within a few days. Meanwhile, the object has been detected at ultraviolet, radio and, very tentatively, X-ray wavelengths and has been granted the inspiring title 1987a.

The neutrino detection was announced by C. Castagnoli and other members of an Italian/Soviet collaboration working at the Mont Blanc neutrino observatory. Five neutrino pulses of energies above 7 MeV were observed at 0300 Universal Time on 23 February — nine hours before the first optical detection.

Happily, and for the first time, it has also been possible to pinpoint the star whose demise has so brightened the lives of astronomers. The object appears to be the blue B-type supergiant Senduleak −69 202 with a mass between 30 and 50 times that of the Sun. This identification agrees well with the notion that, at the end of their nuclear-burning lifetimes, stars of more than a few solar masses undergo a catastrophic transition, shedding much if not all of themselves in the form of a rapidly expanding shell of gas and often, if not always, leaving behind a compact remnant such as a neutron star. Although there are many such stars in our Galaxy, their explosive ends, reckoned to occur on the average once every 50 years, are obscured — like many of the interesting features of the Galaxy — by gas and dust. Thus astronomers have generally had to make do with such explosions detected in other galaxies.

The new supernova is sufficiently close to offer the prospect of resolving the expanding shell within only weeks of the outburst, although the maximum angle of ten milliseconds of arc that will be subtended by the bright photosphere — the deepest part of the shell visible — comes tantalizingly close to the limits even of speckle interferometry. According to Paul Murdin, of the Royal Greenwich Observatory (RGO) in Sussex, spectral lines of hydrogen and helium have been detected from the shell, which is expanding at 16,000 km s^{-1}, but the wide spread of relative gas velocities is for the time being smearing out most of the interesting spectral features beyond recognition. This is no doubt particularly frustrating for those studying a phenomenon that makes supernovae unique and crucial for an understanding of the chemical evolution of the Galaxy — the ejection of heavy elements into the interstellar medium, including not only the residues of the star's nuclear cycle but also products such as short-lived isotopes of nickel and cobalt and stable heavy elements synthesized only during such explosions. Clearly, the neutrino detection is particularly exciting in this context.

Meanwhile, intriguingly, nobody has yet convincingly shown theoretically that supernovae should occur at all. Everybody seems happy that the inert iron core left at the end of a star's nuclear life should, if the star is sufficiently massive, undergo a phase during which electrons are captured by nuclei, thereby removing the capacity of the core to resist the weight of the overlying stellar material and producing a rapid implosion. The core soon reaches nuclear densities and the implosion stops, but nobody has been able quantitatively to explain what happens next. The hand-waving idea is that a shock-wave propagates outwards and expels the outer layers. Specifically, according to Martin Rees of the Institute of Astronomy in Cambridge, neutrinos are released which should be the agents of such disruption, but their mean free paths are too long to be modelled as 'simple' shocks. Some calculations end with a black hole into which the entire star collapses, while others predict a neutron star remnant with outer gaseous layers whose behaviour cannot be well predicted.

What all this means in practice is that everyone is going to want to see what 1987a leaves behind. A hot neutron star would be detectable by its emitted X-rays, although for the first few months these would be obscured by emissions from the scattered energetic particles within the shell. Moreover, according to X-ray astronomers working with the Astro-C/Ginga satellite, observations will be greatly complicated by the nearby bright X-ray source LMC X-1.

The dynamics as well as the nature of the object left behind will absorb much attention — if a neutron star, will it have a high transverse velocity and will it rotate so rapidly as to act as a pulsar?

As if all this were not compelling enough, the supernova also provides an opportunity to explore the dynamics and the history of the material lying along the line of sight. Already, according to Max Pettini of the RGO, absorption lines of calcium, sodium and magnesium have revealed at least twenty distinct gas motions, many within the LMC and others within our own Galaxy. But the brightness of the object should also allow the detection of comparatively weak absorption features due to trace isotopes whose abundances constrain theories of primordial nucleosynthesis (such as ^{7}Li) or galactic chemical evolution (such as ^{13}C/^{12}C). Previous studies of this kind have been confined to the region within 300 light years or so of the Sun, but now there seems to be a prospect of similar observations within the LMC. The point is especially intriguing because the LMC is thought to be chemically much younger than our Galaxy.

So most astronomers are understandably delighted, but a small sub-group of them, doubtless now impotently furious, has already missed its only chance to exploit this event. The most aspherical type of core implosion to produce 1987a would also have generated gravitational waves. Maddeningly, no cryogenic detectors were operating but two bar detectors at the University of Maryland were taking data yet to be examined. **Philip Campbell**

Radioastronomy

A new signpost in the sky

Ray P. Norris

SINCE the discovery in 1951 of radio emission from neutral hydrogen in our Galaxy, radioastronomers have persistently been seeking other molecules, and additional transitions of detected molecules, with which to probe the interstellar medium. Recent rapid advances in receiver technology have prompted an ever-increasing rate of discoveries of new transitions, so that nowadays the detection of a new transition rarely attracts much attention. The discovery of a new methanol maser, reported by W. Batrla, H.E. Matthews, K.M. Menten and C.M. Walmsley on page 49 of this issue, is surely an exception to this trend of complacency. Batrla *et al.* have discovered not just another weak transition of a well-known molecule, but a source of strong maser action whose intensity rivals that of the well-known OH and water-vapour masers (Elitzur, M. *Rev. Mod. Phys.* **54**, 1225–1229; 1982). The significance of their result lies not so much in its immediate astrophysical implications, but in its implications for the observational study of star formation.

OH and water-vapour masers have been studied extensively since their discovery in the late 1960s in regions of star formation and around old, dying stars. In only a few cases, such as the OH emission from OH infrared stars, do we have any understanding of the inversion mechanisms that must be present to cause the maser action. Despite this staggering ignorance, these masers have turned out to be powerful tools for radioastronomers, primarily because of their sensitivity to physical conditions in the surrounding gas. Observations of masers can, in principle, reveal something of the molecular abundance, density, velocity, temperature and magnetic field. In addition, their high intensity and small size renders them easily accessible to radio telescopes (Norris, R.P., Diamond, P.J. & Booth, R.S. *Nature* **299**, 131–134; 1982). Thus, OH and water-vapour masers stand as signposts in the swirling interstellar gas cloud, indicators of the conditions and kinematics of the gas around very young and very old stars. It is fortunate that the OH and water-vapour masers occur in different regions of the gas, allowing two different regions to be probed.

Batrla *et al.* now add a third type of signpost. Unlike the other, weaker maser transitions of methanol and formaldehyde, the new methanol masers are strong enough to be studied in fine detail by radio synthesis telescopes, and their spectra show structure that promise the complex detail familiar from OH and water-vapour observations. Because the methanol masers probably occupy a different regime from the OH and water-vapour masers, they offer a chance to add pieces to some very sparsely filled jigsaws.

The sources in which the methanol masers were discovered are the old faithfuls of the OH and water-vapour literature. Each is a known region of active star formation in which OH and water-vapour masers have already been found. The search will now be on for methanol masers in other types of object. Extragalactic methanol masers will no doubt soon feature in these pages, and diverse types of object, ranging from comets to supernova remnants, will be fair game for maser-hunters.

These methanol masers suffer, however, from one major drawback. They have been discovered too late for the radioastronomy lobby to try to safeguard (as was done for hydrogen atoms and OH molecules) that portion of the radio-frequency spectrum in which they lie. Symptomatically, Batrla *et al.* were unable to study several sources because of satellite interference. By the same token, few radioastronomy telescopes will be equipped to study the frequency of this methanol transition (12.179 GHz), even though it is technically very accessible. One hope lies in the common availability for this band of commercial satellite receivers, which could easily and cheaply be fitted to existing radioastronomy antennas. Whether this will actually happen will depend on the degree of pressure which interstellar astronomers are able or willing to apply to their engineering and administrative colleagues, and the extent to which such observations would be sabotaged by satellite interference. □

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Protein evolution

Positively darwinian molecules?

Andrew Leigh Brown

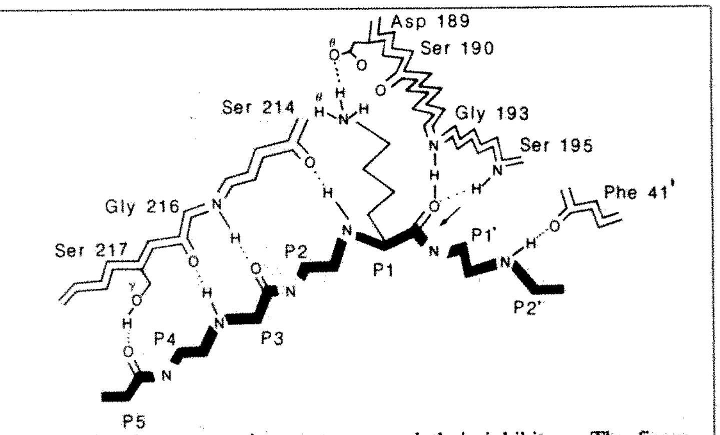
ACCORDING to the neutral theory of molecular evolution, almost all nucleotide substitutions that are fixed during evolution are selectively neutral, or effectively so. That is, the absolute value of s, the selection coefficient, is much less than $1/2N_e$, where N_e is the effective population size[1]. It is now well known that synonymous nucleotide substitutions, which do not lead to an amino-acid change, occur at least 2–3 times more rapidly than those substitutions that do cause such a change, during the evolution of most proteins. In addition, in pseudogenes (defective coding sequences sometimes found in multigene families) the rate of non-synonymous substitution accelerates until it equals the synonymous rate. Thus, at the molecular level, rapid evolution has become associated with the lack of any functional difference between substituted alleles. But exceptions to such generalizations can often be more interesting than the rule. Two recent studies[2,3], one reported by R.E. Hill and N.D. Hastie on page 96 of this issue[2], provide just such an exceptional case.

Serine proteinase inhibitors are a large group of proteins found in many prokaryotes and eukaryotes. Several families have apparently evolved the inhibitory properties independently, and the interaction of these inhibitors with their target proteinases has been studied in considerable detail (see ref. 4 for a review). A region sometimes called the reactive centre, which may be duplicated within the molecule, interacts with the active site of the enzyme. One peptide bond within this region is the target for cleavage (see figure). Cleavage occurs extremely slowly, however, and the cleaved products also form a very stable complex with the enzyme. The result is a series of very effective inhibitor molecules. The biochemistry of inhibition is fairly well known for all such inhibitors and so is the precise physiological role of some: for example, controlling the multiple plasma proteinases of proteolytic cascades such as blood clotting. But there are many serine proteinase inhibitors whose physiological role is unknown.

Among the serine proteinase inhibitors of mammalian blood plasma[2] and of ovomucoids (serine proteinase inhibitors isolated from egg-white of birds)[3], the protein sequence of the reactive centre region has undergone much more rapid evolution than the rest of the molecule. As it is known from *in vitro* biochemical studies that amino-acid substitutions in this region can completely alter the substrate specificity of the inhibitor, the biochemical function of these inhibitors must be evolving rapidly. One of the most remarkable features of these data is the way in which the acceleration of the rate of evolution is confined to the functionally active region. If our current understanding of the function of these molecules is correct, then these results lead almost inescapably to the conclusion that changes in the amino-acid sequence of the reactive centre regions have been selected for.

During attempts to unravel the complexities of the array of serine proteinase inhibitors found in mammalian blood plasma, it had earlier been found[5] that the gene encoding mouse contrapsin, an inhibitor active against trypsin and similar proteinases, was most closely similar in sequence to a gene in humans that encodes an inhibitor with a quite different specificity: α_1-anti-chymotrypsin. Similarly, early studies[6] on ovomucoid had shown that the substrate specificity for this inhibitor could be quite different in closely related species. Now, for both gene families, more extensive evolutionary comparisons have been completed.

Contrapsin is now known to belong to a multigene family that encodes at least two classes of messenger (m) RNA. Hill and Hastie in this issue[2] show that a 1.8-kilobase (kb) mRNA that encodes contrapsin in the mouse is also present in the rat. On the basis of the pattern of expression and on DNA sequence similarity, these mRNAs are probably encoded by the equivalent gene in the two species. A 2.2-kb mRNA from this gene family is also present in both species, and is probably equivalent in each, on similar grounds. Hill and Hastie located the reactive centre region of the contrapsin molecule by aligning the DNA sequence with human α_1-anti-chymotrypsin. To consider the evolution of these sequences and their human homologue in detail, the nucleotide sequences can be divided into three regions: the 5′ coding region (nucleotides 1–515, codon 175); the reactive centre (nucleotides 516–573, codons 176–191); and the 3′ region following the reactive centre. There are stretches of considerable similarity between the sequences of all the genes examined, particularly in the 5′ region. Here there are stretches of almost 20 amino acids that have hardly diverged among all four sequences (mouse contrapsin, rat 1.8-kb sequence, rat 2.2-kb sequence and human α_1-anti-chymotrypsin). The 3′ region also shows considerable similarity among these sequences at both the nucleotide and amino-acid level. But there is a remarkable degree of divergence in the reactive centre region. In only one codon out of sixteen is the same amino acid found in more than two of these sequences. Analysis of the relative rate of synonymous versus non-synonymous substitutions is also revealing. In region 1, the 5′ end of the gene, the ratio resembles that of globin genes with synonymous nucleotide substitutions occurring 2–3 times faster than replacement substitutions. Within the reactive centre, however, the frequency of replacement substitutions per replacement site rises to equal the frequency of synonymous substitutions per synonymous site in the 3′ region and to exceed that for the 5′ coding region. Remarkably, the extent of the region of divergence exactly coincides with the reactive centre, which in fact is flanked by fairly well-conserved sequences.



Interaction between serine proteases and their inhibitors. The figure shows the main contact region in the predicted complex between trypsin and Japanese quail ovomucoid. Residues of the reactive centre region of the inhibitor are labelled P1 to P5, P1′ and P2′. The cleavage target peptide bond is marked with an arrow. (From ref. 8.)

In the case of ovomucoids, there can be up to three domains containing reactive centres, and in some cases these have evolved different specificities within the same molecule. Laskowski and colleagues have now obtained[3] the amino-acid sequence of the third domain of ovomucoid from 101 species of birds. Although detailed analysis of the rates of evolution of these sequences depends on the phylogenetic framework within which the data are interpreted, and this is currently a topic of some debate, it is nevertheless clear that the distribution of amino-acid substitutions along the 51-residue sequence is quite unlike that found in almost all other proteins. In particular, when I examined data from ref. 3, I found the mean number of amino-acid differences in this collection of sequences at those sites which contact the proteinase to be almost double the mean for the whole domain — a statistically highly significant difference.

There is no doubt about the biochemical importance of the highly variable residues in these serine proteinase inhibitors, especially for the ovomucoids where the enzyme–inhibitor complex has been studied directly by X-ray crystallography. Mechanisms for generating sequence variation other than nucleotide substitution, such as gene conversion, can be excluded in both cases because of the striking difference in the rate of evolution of residues in the reactive centre and their immediate neighbours which do not contact the proteinase.

The simplest explanation for these observations seems therefore to be that new amino-acid sequences in the reactive centre regions have from time to time been favoured by natural selection (and so s is positive). But in neither case is the potential selective agent known. The widespread occurrence of serine proteinases makes this a difficult task. Hill and Hastie suggest[2] that the substrates for contrapsin-like molecules are exogenous proteinases synthesized during the course of infection by parasites. This is an interesting proposal as it offers an immediate explanation for the high rate of change. As Haldane originally pointed out[7], specific host–parasite interactions of this type can result in frequency-dependent selection favouring the maintenance of several alleles of the proteinase of the invading parasite with different specificities in the reactive site. Each new allele of the parasite enzyme would generate a new selective challenge to the host — an evolutionary 'race' begins. Although other explanations have been postulated[3] they do not contain this powerful force for the generation of variability. ☐

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100 years ago

Some years ago I was about to drown a terrier pup of about a month old. I held it across the palm of my open hand over a large tub of water. It lay quite still on my hand as I gently lowered it. When within 4 inches of the surface, but not yet touching the water, it deliberately began, and continued as long as I held it there, the paddling motion with its feet peculiar to dogs when swimming, and quite unlike that of walking, although I am perfectly certain this puppy had never seen or touched water before. We know almost all animals swim when first placed in water, but how could this puppy know before it touched the water that this pecular action would be necessary? Has a similar case been observed by any of your readers?

Birmingham, February 17 D.W.C.

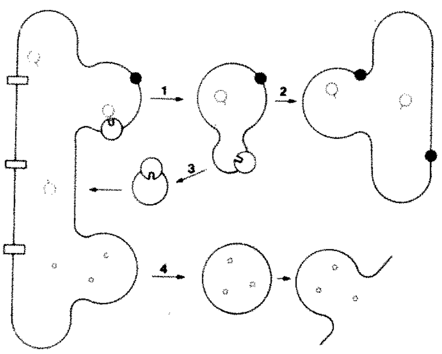
From *Nature* **35**, 392; 24 February 1887.

Protein transport

From organelle to organelle

Regis B. Kelly

DOMAINS that determine where a protein will be located in the cell, as opposed to what it will do, are called sorting domains (see figure). The domain specifying delivery from the Golgi complex to the vacuole in yeast cells has now been shown[1,2] to be a short contiguous sequence of amino acids at the amino terminus of the protein. Although contiguous amino-acid sequences are known to direct proteins from the cytoplasm to intracellular organelles such as the endoplasmic reticulum or the mitochondria, this is the first example of a sorting domain that specifies movement between organelles. R. Anderson and colleagues, in a separate set of observations reported in ref. 3 and on page 77 of this issue[4], find that the lumen of Golgi-associated vesicles is most acidic in the region where newly synthesized secretory proteins leave the Golgi area for mature secretory vesicles. The observations give further support to the notion that unidirectional flow of proteins from one organelle to another involves *p*H-sensitive receptors. Yeast-cell mutants deficient in interorganelle sorting[1,5] may allow direct testing of such sorting hypotheses.



Steps in organelle-to-organelle sorting. Any newly synthesized protein destined either for secretion or for one of the membrane-bounded compartments of the cell is directed first to the Golgi complex. In the Golgi complex, the proteins are sorted, by unknown mechanisms, to their correct intracellular destinations. If a newly synthesized protein lacks sorting information it leaves the Golgi complex and goes directly to the surface of the cell by a default pathway. Proteins destined for delivery to the lysosomes and, in certain secretory cells, for delivery to the secretory granule may be sorted out of the default pathway by the above sequence of steps. (A parallel sequence of steps is hypothesized for delivery of proteins from the cell surface to the endosomes.) 1, Membrane proteins, including those that are receptors for ligands with sorting domains, are segregated away from resident membrane proteins of the donor organelle. 2, In a compartment with an acidic interior, ligands dissociate from receptors. Free ligands are targeted to the donor organelle. 3, Empty receptors are segregated from the other membrane proteins and soluble ligands and return to the donor organelle. 4, Soluble proteins that lack sorting domains can escape by a non-selective default pathway. If steps 2 and 3 are blocked with an acidotropic drug, ligands with sorting domains exit through the default pathway.

Mammalian cells most often use a mannose phosphate receptor to target newly synthesized lysosomal enzymes to the lysosome[6]. Yeast cells use a simpler sorting mechanism, that does not require carbohydrate, to target digestive enzymes to the vacuole, an organelle analogous to the lysosome of animal cells. When as few as 50 amino acids from the amino terminus of procarboxypeptidase Y, a vacuolar enzyme precursor, are fused to the amino terminus of invertase, a secreted protein, the hybrid protein goes to the vacuole[1]. Mutational studies[2] show that the first 10 amino acids at the amino terminus of procarboxypeptidase are sufficient to specify the location of cell vacuoles. When this sequence is mutated, or the sorting machinery is defective, vacuolar enzymes escape from the cell by the pathway normally taken by secreted proteins. Because no sorting information is apparently required for this secretion pathway, computer-friendly biologists now call it the default pathway. Work in the laboratories of Stevens (University of Oregon, Eugene)[5] and of Emr (Caltech)[1] has made elegant use of the diversion of mis-sorted vacuolar enzymes to the default pathway to identify at least 14 complementation groups involved in targeting vacuolar enzymes. Such mutations need not affect vacuolar membrane protein localization (J. Rothman and T. Stevens, personal communication), implying that sorting of membrane and soluble vacuolar proteins involves at least partially different mechanisms.

What molecular machinery is required to transfer vacuolar enzymes from Golgi complex to vacuole? A good bet is that the sorting machinery will use acidity differences between intracellular organelles. Acidity differences have now been discovered[3,4] between regions of the Golgi complex in at least two cell types that sort secreted proteins into the regulated secretory pathway. The key advance in these studies is the use of a novel membrane-permeable acidotropic molecule, 3-(2,4-nitroanilino)-3′amino-*N*-methyldipropylamine (DAMP). This molecule accumulates inside acidic compartments in the cell, and has a dinitrophenol (DNP) group that can be recognized by DNP-specific antibodies. The molecule also has an amino group that allows gluteraldehyde fixation. By quantifying the binding of anti-DNP using gold-conjugated protein A or secondary antibodies, it is possible to make a semi-quantitative measurement of the *p*H of intracellular organelles. In insulin-secreting endocrine cells, the condensing vacuoles to which proteins are delivered immediately on leaving the Golgi cisternae are much more acidic than the cisternae themselves[3]. Because low *p*H facilitates the proteolytic conversion of proinsulin to insulin, acidification in this case could be necessary solely to ensure that proteolysis is a late, post-Golgi event. Exocrine cells, however, do not require proteolysis of their secretory granule enzymes and, unlike endocrine cells, they do not have secretory granules with a low internal *p*H. When Orci and colleagues examined exocrine cells with DAMP, as they report in this issue[4], the secretory granules are not acidic as expected, but the same acidification increase is seen from Golgi cisternae to immature secretory granule.

Several cellular processes could be responsive to a *p*H gradient from the Golgi complex to the immature vesicles of regulated secretory cells. The hypothesis with perhaps the strongest appeal to cell biologists is that the *p*H gradient allows vectorial, one-way traffic of secreted proteins from the Golgi complex to immature secretory granules. In this hypothesis, vectorial sorting into the regulated, secretory pathway would parallel vectorial delivery of ligands by receptor-mediated endocytosis[7], and delivery of newly synthesized lysosomal enzymes from Golgi lysosome to lysosomal precursor[6]. To achieve vectorial transport during endocytosis and lysosome biogenesis, receptors carry ligands from a donor organelle to an acceptor organelle where the acidic interior promotes ligand dissociation (see figure). The empty receptors then recycle to the donor membrane while the ligand proceeds to the lysosome. Energy that is needed to ensure vectorial one-way traffic is provided by the ATP-driven proton translocases that generate the *p*H gradient. Because the mannose phosphate receptor and receptors involved in receptor-mediated endocytosis show *p*H-dependent ligand association, acidotropic drugs which destroy *p*H gradients block vectorial delivery. An old observation[8] suggested that delivery of hormone to secretory granules in endocrine cells was similarly blocked by acidotropic molecules. This result and that showing the accumulation of DAMP in immature secretory granules are consistent with a model in which a *p*H-sensitive, recycling receptor executes one-way vectorial traffic of endocrine hormones and zymogens into their respective secretory organelles. It would be very exciting if vacuolar sorting in yeast cells, where mutants are now available, uses the same basic *p*H-sensitive recycling mechanism, and perhaps also clathrin-coated segregation of receptors, to sort newly synthesized proteins out of the default pathway. If so, cell biologists will have yet another unifying hypothesis to simplify their lives. ☐

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Mid-ocean ridges

A magma chamber observed?

Charles H. Langmuir

A SUBSTANTIAL step towards clarification of the controversy about magma chambers has been taken in the past year by a team of investigators from three US oceanographic institutions who carried out a comprehensive multi-channel seismic survey of the East Pacific Rise. On page 35 of this issue[1], Detrick *et al.* report the preliminary results of the survey. These results constrain for the first time the distribution of magma chambers along the East Pacific Rise, and provide the best constraints yet on the width of the axial magma chamber.

The concept of a magma chamber, where magma is stored before its eruption from volcanoes, has been central to petrological thought for much of this century. The concept is particularly attractive for the 50,000-km-long string of volcanoes that make up the ocean ridges, where the plates of the Earth spread apart at rates between 1 and 20 cm per year. Magma rises from the mantle to fill the crack created by spreading, and this continual supply of magma has been suggested to supply a large 'steady-state' magma chamber that would exist perpetually along the global system of ocean ridges. This model has been called the 'infinite onion' model of ocean ridges[2], because the magma chamber peels off solid layers at its edges while continually growing from its centre. Scientists studying ophiolites, old ocean crust exposed on land, have suggested that ocean ridge magma chambers can be as much as 30 km wide, in apparent agreement with the infinite onion model[3].

But there has been a contradiction between this conceptual model of ocean ridges with large, steady-state magma chambers and the actual evidence from geophysical and geochemical studies. Indeed, some have suggested, on the basis of both petrology[4-6] and geophysics[6,7], that magma chambers are small and ephemeral rather than large and steady-state.

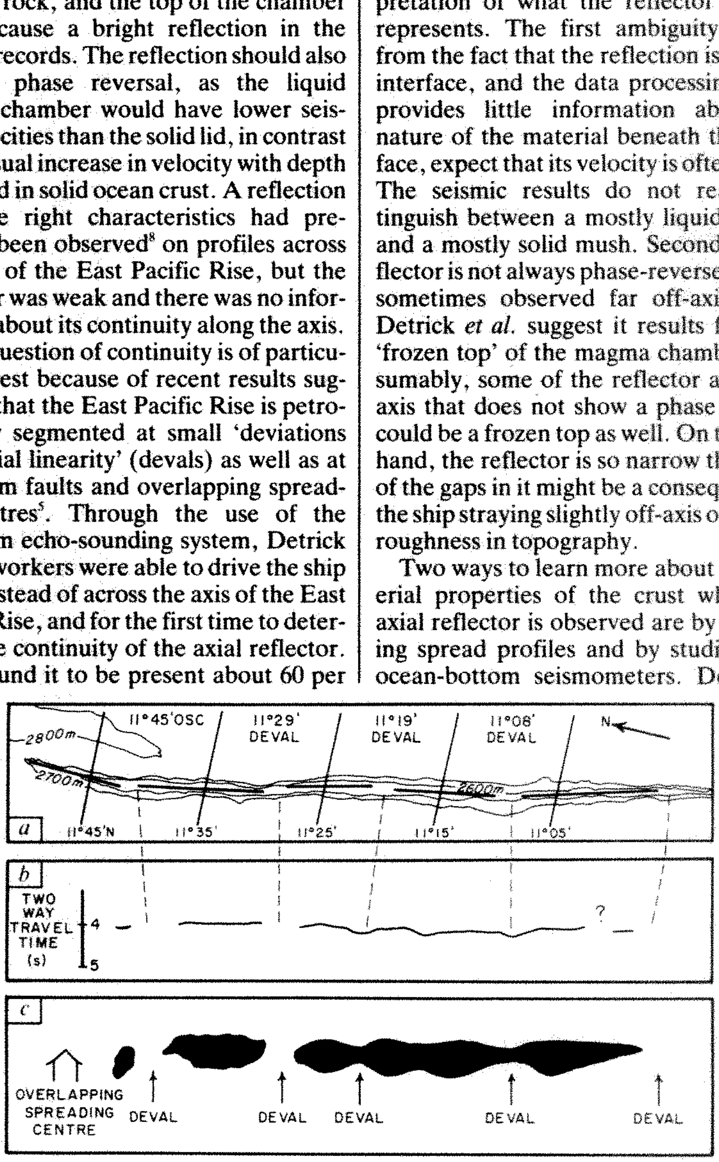
The multi-channel technique reveals reflecting horizons within the ocean crust. A magma chamber would be a zone of low-velocity liquid surrounded by higher-velocity rock, and the top of the chamber should cause a bright reflection in the seismic records. The reflection should also show a phase reversal, as the liquid magma chamber would have lower seismic velocities than the solid lid, in contrast to the usual increase in velocity with depth observed in solid ocean crust. A reflection with the right characteristics had previously been observed[8] on profiles across the axis of the East Pacific Rise, but the reflector was weak and there was no information about its continuity along the axis.

The question of continuity is of particular interest because of recent results suggesting that the East Pacific Rise is petrologically segmented at small 'deviations from axial linearity' (devals) as well as at transform faults and overlapping spreading centres[5]. Through the use of the SeaBeam echo-sounding system, Detrick and co-workers were able to drive the ship along instead of across the axis of the East Pacific Rise, and for the first time to determine the continuity of the axial reflector. They found it to be present about 60 per cent of the time, and the width of the reflector, where present, usually to be less than 3–4 km. The reflector is discontinuous across overlapping spreading centres and transform faults, and in some (but not all) cases is discontinuous or shows marked dips at devals.

It is tempting to associate the axial reflector with the magma chamber in the infinite onion model. If the reflector is a magma chamber, then the seismic data imply that the magma chamber is an order of magnitude smaller than the ophiolite-based model. Also, because the magma chamber is present beneath only a little more than half of the length of the East Pacific Rise studied, it cannot be a steady-state feature except possibly in limited areas.

Several ambiguities remain in the interpretation of what the reflector actually represents. The first ambiguity derives from the fact that the reflection is from an interface, and the data processing so far provides little information about the nature of the material beneath the interface, expect that its velocity is often lower. The seismic results do not really distinguish between a mostly liquid magma and a mostly solid mush. Second, the reflector is not always phase-reversed, and is sometimes observed far off-axis where Detrick *et al.* suggest it results from the 'frozen top' of the magma chamber. Presumably, some of the reflector along the axis that does not show a phase reversal could be a frozen top as well. On the other hand, the reflector is so narrow that some of the gaps in it might be a consequence of the ship straying slightly off-axis or of local roughness in topography.

Two ways to learn more about the material properties of the crust where the axial reflector is observed are by expanding spread profiles and by studies using ocean-bottom seismometers. Detrick *et*



Possible distribution of magma chambers along the East Pacific Rise. *a*, Bathymetry of the East Pacific Rise in plan view between 10°50'N and 1°45'N[1]. The Clippeton transform fault is another 60 km to the south, at 10°15'N. Bold straight lines indicate individual volcanic segments separated by devals. *b*, Depth (in seconds of two-way travel time) and location of the axial reflector indentified by the seismic survey[1]. Devals in the bathymetry and corresponding gaps or dips in the reflector are connected by the vertical dashed lines. *c*, Vertical section of the possible distribution of magma chambers beneath this portion of the East Pacific Rise. The chamber is discontinuous across spreading centres, and may thin or disappear across devals.

al.[1] carried out one expanding spread profile on the axis within the region where they report along-axis data, and there is evidence for an extensive zone of low velocity at that one point along the axis. But both axial mush and an axial magma chamber could produce such a low-velocity zone.

Studies using ocean-bottom seismometers potentially hold great promise as a complement to the multi-channel seismic studies, because they can evaluate waves which pass through the low-velocity zone rather than waves that are reflected off it. Bratt and Solomon[7] reported the analysis of seismograms from a three-component ocean-bottom seismometer at 11°20′N, where Detrick *et al.*[1] found there to be a weak axial reflector in the multi-channel seismic data, and where there is a deval[5]. Bratt and Solomon concluded that any magma within the crust in this region must be confined to narrow dykes or small isolated bodies with less than 1 km vertical thickness. Thus, even in regions where the seismic reflector is continuous there may be variations in the crystallinity and thickness of the axial low-velocity zone. An along-axis profile that might satisfy the seismic constraints and be consistent with the inferences from the petrological data is sketched in the figure. Given the new along-strike perspective of the multi-channel data, new ocean-bottom seismometer studies located where the axial reflector is strongest and where it is absent should help to clarify the relationships among the multi-channel seismic reflector, possible axial magma chambers and the chemical systematics of basalts erupted at the East Pacific Rise. □

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Cell motility

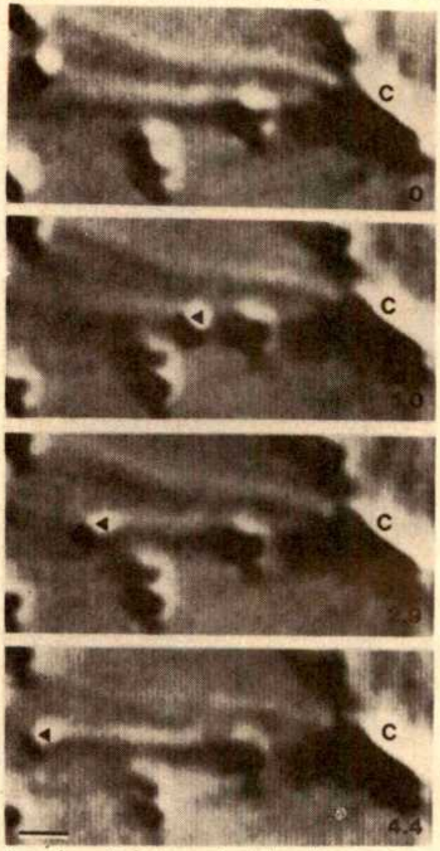
Microtubule activation of kinesin ATPase activity

Stanley Cohn

UNDERSTANDING the molecular mechanisms responsible for force generation along cytoskeletal elements such as microtubules and actin filaments is central to the study of intracellular movement of organelles. Several proteins have been isolated from eukaryotic cells that interact with these cytoskeletal elements and are found to use ATP to generate motile force. The best-characterized of these proteins, myosin, hydrolyses ATP at a rate directly coupled to motile activity. Thus, the ATPase of purified myosin is low, but is several hundred times higher in the presence of actin filaments[1]. Although purified dynein, another mechanochemical protein, has a substantial rate of ATP hydrolysis, it has recently been shown[2] that this activity can be stimulated by microtubules, the cytoskeletal cofactor of dynein. And new evidence[3] obtained by Kuznetsov and Gelfand shows that kinesin, another force-transducing protein, hydrolyses ATP at rates appropriate for organelle transport along microtubules.

Kinesin has recently been isolated from several cell types where it may participate in organelle transport and mitosis (refs 4,5; see ref. 6 for review). Kinesin has been shown by video-enhanced microscope assay to be capable of producing translocation of carboxylated latex beads along microtubules or of microtubules over a glass coverslip (see figure). The motility induced by neuronal and sea urchin egg kinesin is totally dependent on the presence of hydrolysable nucleotides, with a maximal translocation velocity of approximately 0.5 μm s^{-1} when in saturating (> 100 μM) concentrations of ATP[7,8]. Assuming that each kinesin crossbridge translocates a microtubule or bead a distance of one to two microtubule subunits (about 10 nm), then an active kinesin molecule would be expected to hydrolyse ATP at a rate of around 50 per second, a value that would be similar to the activity of the actin-activated MgATPase of myosin 'crossbridges' (subfragment-1) and that of the microtubule-activated ATPase of dynein. However, both neuronal and sea urchin kinesin, when prepared by a procedure based on the tight binding of kinesin to microtubules in the presence of the unhydrolysable ATP analogue AMPPNP and its subsequent release by ATP[4–6], do not possess these predicted high rates of ATPase activity.

In their new work, Kuznetsov and Gelfand[3] obtained preparations of bovine brain kinesin that hydrolyse ATP at the high rates predicted from the motility assays. Interestingly, the authors imply that kinesin prepared by the method of binding kinesin to microtubules in the presence of AMPPNP yield fractions with low ATPase activity. But if they replace AMPPNP with inorganic tripolyphosphate (PPP_i) in the microtubule-affinity step, they obtain material on ATP release which elutes from a gel-filtration column as a protein complex containing a major polypeptide of relative molecular mass (M_r) 135,000 and minor ones of M_r 45,000–70,000. This protein complex hydrolyses MgATP at a rate of 60–80 nmol min^{-1} mg^{-1} and is stimulated about 60-fold to 4.6 μmol min^{-1} mg^{-1} in the presence of microtubules.



A series of photographs taken from a time-lapse video sequence showing the distally oriented movement of a kinesin-coated bead (marked with a triangle) along a microtubule which had been nucleated and assembled off an isolated centrosome (C); lower right-hand numbers are seconds after bead attachment. Scale bar, 1 μm. For details see refs 7 and 12.

Although Kuznetsov and Gelfand did not directly study the motility-inducing activity of their preparations, there are several reasons for believing that their microtubule-activated ATPase represents the activity caused by kinesin-induced motility. For example, the ATPase activity varies with ATP concentration according to Michaelis–Menten kinetics, with a K_m (the concentration of substrate required for half-maximal activity) for ATP of 12–14 μM. The rate of kinesin-induced microtubule gliding over glass coverslips exhibits a similar variation in this range, resulting in half-maximal velocity at 10–20 μM[7,8]. Furthermore, PPP_i and AMPPNP, both of which promote stable microtubule–kinesin complexes and inhibit kinesin-induced microtubule gliding, were found by Kuznetsov and Gelfand to be competitive inhibitors of the microtubule-activated ATPase. Vanadate and *N*-ethylmaleimide, which are weak inhibitors of kinesin-induced motility[4,8], are also found to be weak inhibitors of the ATPase activity.

Nonetheless, the implication that the use of AMPPNP in kinesin purification yields protein with inhibited enzymatic activity raises some puzzling questions. For example, the microtubule motility promoted by both squid neuronal and sea urchin egg kinesin purified by the AMPPNP-induced microtubule-binding procedure seems to be very similar to the motility observed using crude preparations that have not been exposed to AMPPNP[4,8,9]. If the ATPase is indeed caused by the kinesin microtubule cross-bridge cycle coupled to motility, then it is difficult to understand how the two kinesin preparations (using PPP$_i$ and AMPPNP for microtubule binding), having equal ability to induce microtubule translocation, could differ so greatly in their ATPase activities. Also, AMPPNP, like PPP$_i$, appears to act as a competitive inhibitor of the microtubule-activated ATPase of kinesin, implying that its inhibitory effects should be reversible with increasing ATP concentration. Moreover, Brady and co-workers[10,11] have prepared microtubules from chick and bovine brains in AMPPNP which contain polypeptides of M_r 130,000 and 124,000, respectively, and hydrolyse ATP at a much greater rate than microtubules prepared in ATP in which these polypeptides were absent. It is therefore seems likely that other factors, such as removal of ATPase inhibitors or retention of other regulatory cofactors during purification or the exact conditions of their ATPase assay, also contribute to Kuznetsov and Gelfand's success in isolating enzymatically active kinesin.

The work of Kuznetsov and Gelfand is a significant step in the study of kinesin-induced motility. Measurements of ATPase activity, together with the video-microscope motility assays, should allow the mechanochemical cycle of kinesin to be studied in greater detail, as well as being extremely useful in the development of probes (for example, antibodies) that inhibit kinesin enzymatic activity and that can be used for investigating biological functions of kinesin *in vivo*. The purification protocol developed by Kuznetsov and Gelfand also represents one other advance: the cost of one gram of PPP$_i$ is about $13 whereas an equivalent amount of AMPPNP costs $1,407. ☐

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History of science

Joseph Fraunhofer (1787–1826) and heterochromatic photometry

Paul L. Pease

JOSEPH Fraunhofer, born 200 years ago on 6 March 1787, was probably the first to use the criterion of a minimally distinct border to equate the brightness of two heterochromatic fields. The purpose of heterochromatic photometry is to determine at what radiance lights of different chromaticity have an equal visual effect. Results obtained with the minimally distinct border and other procedures of heterochromatic photometry are central to our understanding of the inter-relationships of physical, physiological and perceptual variables in the processing of visual information. Although Fraunhofer may not have been the very first to use the minimally distinct border, his contribution in the early part of the nineteenth century has been largely unrecognized.

Fraunhofer published two papers[1,2] in 1823 and 1824. The second of these reports his experiments on the brightness of different colours in the spectrum and his measurements of chromatic aberration of the eye. Although Fraunhofer is best known for his contribution to optics, I discuss here his measurements of the brightness of the spectrum and the method he used to obtain them. Why did Fraunhofer want to know, as he stated on page 32 of ref. 2, "the intensity of each colour in the spectrum, or in what ratio the impression of any colour of the spectrum is stronger or weaker than that of another colour?" He had worked out the design for achromatic doublets for telescopes that were, of course, intended to be used by the eye. He was interested in certain ocular parameters, such as axial chromatic aberration and spectral sensitivity, and he reasoned that the residual chromatic aberration of an achromatic lens would be bothersome to an observer if any residual aberration occurred in those parts of the visible spectrum that appear the brightest. He wrote on pages 31 and 32 ". . . the aberration of the yellow rays, for example, which have the greatest brightness, will produce in the ratio of their intensity a worse effect than the violet ones, if the aberration for the latter is of the same magnitude".

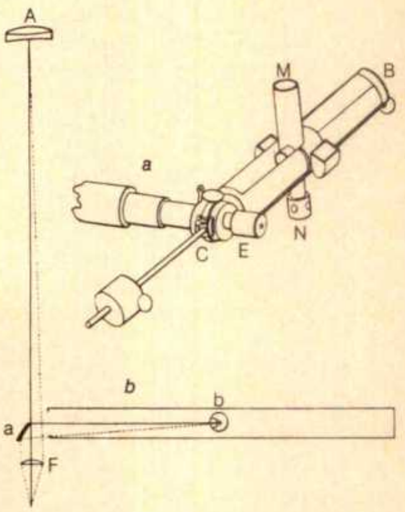

Fig. 1 Two views of Fraunhofer's photometer. *a*, A portion of the modified theodolite. At right-angles to the axis of the eyepiece (E) is a tube (CB) which carries a small flame in a moveable tube (MN). *b*, A is the objective lens which received spectral colours formed by a prism placed before the lens. In the focal plane of the eyepiece (F) is a mirror (a) which was illuminated by light from a flame (b). The mirror divided the field of view in half with a sharp vertical edge (from ref. 2).

To measure the brightness of the different parts of the spectrum, Fraunhofer used a theodolite, a telescope used for the measurement of angles (Fig. 1). The light of different colours of the spectrum of the Sun were obtained by a glass prism and viewed through the telescope. In the focal plane of the ocular, he "applied a small plain metallic mirror, the edge of which being well defined" (page 32). The mirror was placed at 45° to the axis of the telescope that bisected the field of view. A tube at right angles to the ocular contained his standard source — a small aperture placed before an oil flame. He varied the illuminance of the flame at the mirror by changing the distance of the source from the mirror. Fraunhofer had, in fact, constructed a visual photometer with a circular bipartite field, one half illuminated by his standard lamp and the other by the dispersed spectrum of sunlight. Knowing the distance of the lamp from the mirror and applying the inverse square law, he obtained measurements of the relative brightness of different spectral lines. The criterion he used to compare the two halves of the field which, of course, had different chromaticities, was a minimally distinct border. "Though at first it appears difficult to compare the light of two different colours, yet it becomes easy by a little practice. The intensity of the light of the mirror approaches more to that of any colour in the spectrum, if at the same position of the eye-glass its vertical margin is less distinct" (page 32).

Fraunhofer reported the results of four experiments. "Though the experiments were made in clear weather, and at noon, I sometimes perceived, in the course of the observations, a slight change in the density of the light which the prism received. The differences in the four sets of experiments may have been partly owing to this change, and the flame may also have changed its intensity in the course of the observations" (page 33). This, then, is his explanation of why he obtained variable results from one experiment to the other. I emphasize this point here because of Helmholtz's later criticism (see below). Fraunhofer was aware that his results were relative measurements because he noted that the brightness of different parts of the spectrum was dependent on the source of light. He was also well aware that his results could have been influenced by variability in the absolute intensity of either the Sun or the flame.

I next re-examined the 1968 paper[3] of Boynton and Kaiser which I believe to be the first modern account of the minimally distinct border criterion. Were these authors aware of Fraunhofer's contribution? They state "Our search of the literature has found no evidence of such an experiment; nearly all writers refer to an equal-brightness criterion, making no reference to the quality of the border between the fields" (page 368). The authors cite ref. 4 and state: "Helmholtz mentions that Fraunhofer wrote of a minimally distinct border that could be achieved between areas of different color, but Fraunhofer was not concerned with photometric measurement". I believe that two errors should be corrected: first, the statement "Fraunhofer was not concerned with photometric measurement" contradicts Fraunhofer's own reason for measuring the brightness of different colours; and second, Helmholtz and/or Southall[4] erred in saying "The numerical results of Fraunhofer's measurements of the brightness of the different portions of the spectrum agreed very poorly, mainly, no doubt because he was ignorant of the effect of the absolute intensity on the relative luminosity of the colours". On the contrary, Fraunhofer wrote about how variation in the absolute intensity could have affected his results.

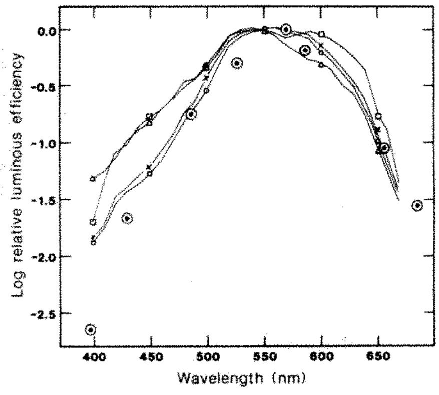

Fig. 2 Mean results of Fraunhofer's four experiments (corrected for the spectral irradiance of the Sun) compared with other measures of relative luminous efficiency (from ref. 7). Symbols: ⊙, Fraunhofer's data; O, heterochromatic flicker photometry; X, minimally distinct border; △, step-by-step; ☐, direct comparison. The last two are measures of direct heterochromatic brightness matching.

It is of interest to know how the results of Fraunhofer's experiments compare with more recent measurements. Leitner has described[5] Fraunhofer's contribution but, like Fraunhofer, did not correct the data for the irradiance of the Sun. I therefore corrected Fraunhofer's data for the abolute irradiance of the Sun according to ref. 6. The corrected measures are shown in Fig. 2 together with more recent heterochromatic data[7]. Apart from the extremes of the spectrum, it is clear that Fraunhofer had it about right.

Certainly the knowledge that Fraunhofer used the minimally distinct border criterion more than 150 years ago does not diminish the significance of the contributions of Boynton and Kaiser and others (see refs 7–9). But the anniversary of Fraunhofer's birth is an appropriate time to set the record straight. ☐

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Protein – DNA interaction

Another folding problem?

Timothy J. Richmond

Now that the first structure of a protein–DNA complex at atomic resolution has been solved by X-ray crystallography and with several other structures on the way, the variety of means by which protein molecules recognize the DNA double helix is becoming clearer. McClarin *et al.* have recently reported[1] the X-ray structure of a bacterial restriction endonuclease, *Eco*RI, bound to a short DNA fragment containing the recognition site of the enzyme. The enzyme does not cleave the DNA because Mg^{2+}, which is essential for catalysis, is left out of the crystallization mixture. Comparison of this structure with that for the free DNA, which is known[2], reveals that the protein induces distortions or kinks in the DNA that are required for formation of the specific complex.

The *Eco*RI protein binds DNA as a dimer, giving the complex a dyad axis of symmetry which coincides with a crystallographic dyad (see figure). The DNA double helix is unwound at this central dyad position by about 25° because of the interactions which are made between its phosphate groups and major groove and the chains extending from two α-helices on each protein monomer. The side chains of one glutamate and two arginines from each subunit make six hydrogen bonds with five base pairs in the hexanucleotide recognition site. Each of these side chains and base pairs participates in two hydrogen bonds. The unwinding of the DNA that permits the formation of all these hydrogen bonds may itself be sequence-dependent. A synthetic homologue of the DNA fragment TCGCGAATTCGCG used by McClarin *et al.* in which the central adenines are replaced with 2,6-aminopurine might provide a test of his hypothesis: it would introduce a base pair that mimics the Watson–Crick hydrogen bonds of G·C while preserving the positions of hydrogen-bond donors and acceptors in the major groove.

The irregular conformation of the DNA centred on the molecular dyad is not a kink in the sense proposed originally[3] by Crick and Klug. These authors suggested that DNA might not bend smoothly to supercoil at the radius of about 45 Å found in the nucleosome, but instead kink at regular intervals between straight segments of B-form double helix. In their model for a kink, contacts between the flat surfaces of neighbouring base pairs were broken and replaced by interactions with protein or water. The DNA seen in the X-ray structure of the nucleosome core particle is actually bent tightly in several places, but the base stacking is not yet clear[4].

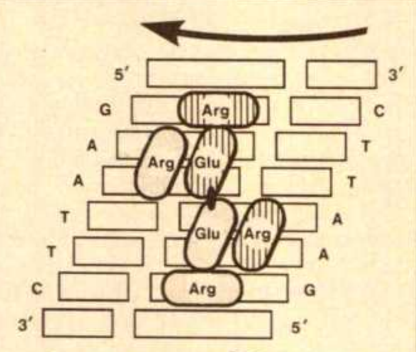
McClarin *et al.* broaden the definition of a kink to include twist as well as curvature distortions of idealized B-form DNA.

Because the DNA fragment on its own does not untwist[2], they call the distortions induced in it by *Eco*RI binding type I and type II 'neokinks'. In contrast to the untwisting that characterizes the type I neokink, the type II neokink, which occurs at the base step flanking the hexanucleotide recognition sequence, is characterized predominately by a bend of 30 ± 10° into the DNA minor grove. When this structure has been fully refined, it might be possible to measure the base-pair unstacking by comparing the difference in the surface accessibility of the DNA in the complex with that for the DNA alone.

The two α-helices in each subunit that contact the DNA are part of a domain in which several α-helices surround a five-stranded β-sheet. The major α-helical regions are oriented with their electropositive amino ends pointing at the negatively charged DNA molecule, compensating for the negatively charged glutamate side chains used in the hydrogen-bonding scheme. A smaller domain, consisting mainly of a two-stranded β-sheet, wraps around the DNA along the major groove. It is required for catalytic activity, but is not essential for specific recognition as it can be removed proteolytically without affecting selective binding. This domain apparently contributes to the formation of the type II neokink, but it is unclear whether this flexing of the DNA phosphate chains is required for catalysis. These details may soon emerge, as Mg^{2+} can be diffused into the crystals to activate the cleavage reaction without disrupting the crystals.

Does the *Eco*RI study have implications for other protein–DNA complexes? The *Eco*RI protein displays a high degree of specificity for its six base-pair recognition site, to which it binds at least 10^4 times more tightly than to random sequences. The *cro* repressor[5], λ-repressor[6], catabolic activator protein[7], *trp* repressor[8] and similar proteins are in another class of highly specific prokaryotic DNA-binding proteins for which X-ray structures are available at high resolution, but lack their DNA counterparts. Model building from the protein structure alone and biochemical experiments could only hint at the DNA contacts responsible for specificity. The low-resolution structure of the 434 repressor–DNA complex suggests that rigid, unbent, B-form DNA, as used in the model-building studies, may not adequately represent high-resolution details[9]. Fortunately, several X-ray investigations of cocrystals containing these proteins bound to DNA and diffracting to atomic resolution are in progress.

All proteins that bind functionally to the DNA double helix show preferences for specific sequences, even though they may not be highly specific like the *Eco*RI protein. Pancreatic DNase I, for example, for which specificity is not a requirement, cuts the phosphate ester bonds of the *Eco*RI oligonucleotide at rates that differ by more than 100-fold[10]. Cleavage of larger DNA substrates[11], and the atomic resolution structure of the DNase I enzyme itself[12], suggest that the discrimination arises largely in the binding step and is caused by the snug fit of a tyrosine side chain into the minor groove of the DNA. The width of the groove is thought to depend on base sequence, but perhaps the enzyme causes additional variations when it binds. Even the nucleosome, which packages more than 90 per cent of the genome of eukaryotic cells, favours certain DNA sequences. On average, a nucleosome will occur roughly every 200 base pairs, but within this length it may be positioned quite precisely, prefering certain sequences and excluding others at distinct locations along the helical ramp formed by the histone core[13]. As much as a 100- to 1,000-fold preference may occur within one nucleosomal repeat. The nucleosome present at the beginning of the sea urchin 5S RNA gene, for example, is particularly well positioned *in vivo* as well as *in vitro*[14,15]. Approximately 160 base pairs are folded around the histone octamer core of the nucleosome; the bending and twisting required to fit the DNA to the protein surface may be energetically more favourable for certain base sequences than for others, such that their combined locations determine nucleosome position and stability. Alternatively, the sequence preference may be controlled locally by the H3 histone dimer in the region where the dyad axis passes through the centre of the DNA[4]. The details of H3–DNA contact are obviously different from those for *Eco*RI, but the twist imparted to the DNA may be similar. Overall, specificity at any level may require sequence-dependent alterations in the DNA structure induced by protein binding.



The hexanucleotide-recognition DNA unwinds to accommodate the hydrogen bonds between it and the *Eco*RI protein. Part of the DNA fragment crystallized with the nuclease is shown schematically, looking into the major groove down the molecular dyad axis. The arrangement of the hydrogen-bonding side chains, three from each subunit of the *Eco*RI dimer, is indicated. The DNA in the complex is twisted and bent substantially[1] compared with the same sequence in the free state[2].

Is the specificity of interactions between protein and DNA governed by simple rules, such as a list of hydrogen-bond match-ups between the two components as was predicted for the 'two-helix motif' proteins, or is the problem comparable in complexity with the protein-folding problem, where a description of the combined intermolecular forces so far eludes us? The answer is apparently yes to both possibilities. The beauty of the X-ray crystallographic solution of the *Eco*RI–DNA complex[1] is that it not only shows the exact mapping of the hydrogen bonds between protein and DNA, but it also reveals substantial distortions of the canonical B-form DNA structure required for the formation of these bonds.

Unravelling the relevant structures by X-ray analysis has dramatically increased our understanding of protein–DNA interactions; there is much more to look forward to. We now know the structures of several types of protein templates to which DNA must conform to be recognized: the restriction enzymes with homology to *Eco*RI; the two-helix motif proteins; pancreatic DNase I; the histone octamer; *Escherichia coli* DNA polymerase 1[15]; and the bacterial chromosomal protein HU[16]. Eventually, knowledge of the structure of DNA-binding protein may allow the prediction of which base sequence will be recognized or, at least within a given class of template, it may be possible to design an altered specificity. On the other hand, the structures of several of the two-helix motif proteins have not yet led to a unique model for the DNA complexes of these systems. Even with the lessons learned from the *Eco*RI–DNA structure, the adventurous model builder lives dangerously — should the DNA be twisted or bent? Perhaps an allosterically regulated DNA-binding protein, such as the *trp* repressor, will provide a few more clues[8]. Comparison of the structures for the non-specific and specific DNA complexes may reveal the limits on how far DNA can twist and bend to conform to a protein. □

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Ozone climatology

More news from Antarctica

Susan Solomon

MUCH effort has been directed towards obtaining a better understanding of the spectacular modification of the Antarctic ozone layer called the ozone 'hole', a precipitous drop in total ozone abundance that has appeared over Antarctica each austral spring (September and October) since about 1975. Forty-five papers on the subject appeared in a special issue of *Geophysical Research Letters* in November 1986. The National Ozone Expedition observational campaign was carried out by 18 scientists (of which I was one) at the National Science Foundation's McMurdo Station during austral spring 1986, and its preliminary results were discussed[1] in a recent News and Views article. The first paper on the results from the expedition, by Hofmann and collaborators, appears on page 59 of this issue[2].

The phenomenon of the ozone hole was first identified[3] by scientists from the British Antarctic Survey, based on a 30-year record of total ozone measurements at Halley Bay, Antarctica. These data suggested[4] that the total ozone concentration should remain rather constant during Antarctic spring, and indeed, such a seasonal cycle was observed[3] before about 1975. The recent data display a different, clearly anomalous, seasonal trend. Further, the magnitude for the spring decline is generally greater from one year to the next, although some year-to-year fluctuations are also observed. Contemporary total ozone abundances in Antarctica in October are only about half as large as those obtained[3] between 1950 and the early 1970s. This is the largest, statistically significant perturbation to a monthly averaged ozone climatology ever recorded.

It is beyond the scope of this article to review the current status of the rapidly growing subject of research on the ozone hole. Instead I will discuss representative rather than comprehensive references. There are several plausible hypotheses[5-8] that seek to explain the observed behaviour of the ozone column. Each results in total ozone changes that are broadly consistent with observations, but each has rather different implications for dynamical tracers; for other chemical species such as nitrogen dioxide, chlorine nitrate or chlorine oxide; and for the vertical structure of the ozone column change. Detailed observations of these parameters and further laboratory studies will be needed before a definitive theory of the ozone change can be advanced. Recent studies provide some interesting insights and apparent contradictions in the behaviour of the Antarctic stratosphere.

It is of great interest to ascertain whether a temperature trend has accompanied the observed ozone trend, because both dynamical and chemical processes (particularly those occurring in polar stratospheric clouds[5,6]) are expected to be closely linked to temperatures. Meteorological analyses[9] based mainly on satellite data display a temperature change as large as 18 °C based on a rather limited time series dating only from 1979 to 1985. On the other hand, a detailed analysis[10] of radiosonde data encompassing the period 1958–1985 suggests a much smaller, but statistically significant trend of about 6–8 °C. The differences between the two datasets are thus quite large and perhaps contradictory, requiring further investigation. Such a trend would be of great importance if substantiated, as it would represent a significant departure from temperature climatology, just as the ozone hole itself is a verified and extreme departure from ozone climatology. Could such a temperature change be unambiguously assigned as the cause, or as an effect, of the observed change in ozone? Radiative transfer theory suggests[11] that the observed change in ozone could lead to a 5–15 °C change, but it seems clear that a significant change in dynamics could yield perturbed temperature and possibly ozone distributions.

A useful database on Antarctic nitrogen dioxide from studies[12] by a New Zealand group, is also available. These data display unusually low abundances during Antarctic spring. Like ozone, the seasonal cycle of nitrogen dioxide is in marked disagreement with conventional theory[5], demonstrating that the chemistry of the Antarctic spring stratosphere is highly anomalous. Further observations of chemical species are needed to determine whether these anomalies are directly associated with the ozone anomaly.

The paper by Hofmann and colleagues in this issue[2] reports a series of ozonesonde observations carried out as part of the National Ozone Expedition. These data provide several important insights into the problem of the ozone hole. Perhaps the most significant is their demonstration that the seasonal decline in the total ozone column is largely confined to the region from about 12 to 20 km. The observed timescale for the ozone change was very short, only about a month. The bulk of the depletion of the total column occurred in September, challenging most of the existing ozone depletion hypotheses[5-8], which can more readily account for a change in October. The total ozone levels in October were affected by substantial increases in ozone near the 24–30-km level, which were associated with planetary wave activity at these upper levels. Year-to-year variations in the October total column abundance may reflect such variations in upper level dynamics even as lower altitudes suffer further depletion.

Also important is the observation[2] of restricted layers in which dramatic depletions of the local ozone abundance had occurred, making important contributions to the integrated column change. The layers were typically a few kilometres in thickness, and many structures repeated on consecutive soundings, demonstrating that they are not an instrumental artefact. On one occasion in October a local depletion of more than 90 per cent was observed in a layer a few kilometres thick at 60 mbar (note that the observed depletion of the integrated column on this date was about 50 per cent). Aerosol measurements on the same flight showed no evidence of corresponding layers that would probably be associated with a dynamical explanation of this phenomenon. On the other hand, previous chemical hypotheses proposed to date do not suggest such large, local ozone depletions. Hofmann *et al.* suggest[2] that the layered structure is associated with chemical reactions following the evaporation of polar stratospheric clouds. These observations may represent the greatest clue and challenge to the mystery of the cause of the ozone hole.

It seems clear that much further research is needed to clarify the cause of this remarkable phenomenon. The Antarctic temperature climatology is of prime importance. Further observations of chemical species, in addition to nitrogen dioxide and ozone, are badly needed, both from the ground and *in situ*. Dynamical tracers such as nitrous oxide and methane should be measured, and laboratory studies of heterogeneous reactions and chlorine chemistry carried out. *In situ* observations of a range of chemical species inside one of the observed layers of spectacular local depletion would undoubtedly add much to the understanding of their origin. □

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Imanishi's influence on evolution theory in Japan

SIR—My Commentary[1] on Imanishi's theory of evolution and the Japanese dimension evoked a gratifying range of responses[2-9]. Several correspondents[3,4] complained about my article being more sociological than scientific. This is indeed the case as my aim was primarily to describe Imanishi's theory and set it in its social context — there is little to be said about its scientific content.

Whereas Nakahara *et al.*[5] claimed that I had rejected Imanishi's theory without fully understanding it, the actual wording of my summary was checked by Imanishi himself who stated "I was very impressed that you have got the ideas very precisely in spite of the language difficulty".

Some of the Western correspondents, such as Sinclair[3], obviously found Imanishi's theory so unusual that they could not believe what I had carefully written. Sinclair states "Imanishi no doubt would not deny that interspecific competition exists", yet that is precisely what Imanishi contends; such denial is the very essence of his theory and it is this which makes it unique. I certainly did not confuse the roles of intraspecific and interspecific competition. Needless to say, I agree that natural selection and the entire edifice of Darwinism requires intraspecific competition. Similarly, I agree with Millar *et al.*[4] that aspects of Imanishi's theory are not unique, such as the denial of the importance of intraspecific competition and his assertion "that the unit for explaining evolution is not the individual but the species". Many of Imanishi's ideas are mirrored in the separate and joint writings of Eldredge and Gould[10-13]. Indeed, Imanishi has intimated his sympathy with much of Gould's writings, and is especially sympathetic to the notion of sudden jumps in evolution, and states "evolution is change occurring when it is time to change".

It is perhaps significant that these selfsame ideas are to be found in the writings of Lysenko[14] (with his own emphasis):

> "No continuous, unbroken series of forms between species — different qualitatively definite states of living matter — have been found. This is so not because the intermediate forms in a continuous range have died out as a result of mutual competition, but because there is no such continuity in nature, nor can there be. *A species is a distinct, qualitatively definite state of living matter.* We must realize that speciation is a transition — in the course of the historical process — from quantitative to qualitative variations. Such a leap is prepared by the vital activity of organic forms themselves, as the result of quantitative accumulation of responses to the action of definite conditions of life, and that is something that can definitely be studied and directed. *The conversion of one species into another takes place by a leap.*".

Interestingly, Japan is one of the few places where Lysenko is still taken seriously. The Japanese Society for Michurin Biology was set up in 1954, the *Japanese Journal of Michurin Biology* was launched in 1965 and still continues in the 1980s. There is clearly a sympathetic response to these ideas in Japan.

My brief discussion on the scientific basis of Imanishi's concepts of protoidentity and habitat segregation or life style partitioning, which I claimed had been effectively refuted, was challenged by Sibatani[2] and Millar *et al.*[4]. The points they raised have been adequately dealt with by Rossiter[6]. I agree that habitat segregation is a real phenomenon, as Nakahara *et al.*[5] insist, but it is not as they or Imanish suppose antithetical to natural selection. The late Kani and Imanishi's close colleague Morisita[15] recognized the importance of interspecific competition. There is no conflict regarding observations of the phenomena. It is in the explanations proposed to account for them that disagreements arise. Hence I agree completely with Sakura *et al.*[8] that "Imanishi's description of phenomena is not incompatible with Darwinian theory which deals with mechanisms of evolution".

Finally, I was pleased to read Sibitani's[2] comments on "Japanese ethnocentric excesses" as well as the letter from the primatologists in Kyoto who say that although they "are still hampered by the influence of Imanishiism, we criticize his theory and try to eliminate its negative influence"[8]. This contrasts with Asquith[9] who challenged me on my general comments on the average intelligent layman's understanding of evolution, which I do not retract.

Darwin's work was indeed introduced into Japan in the last century and in fact social darwinism was one of the cornerstones of the social policy of the rigid authoritarianism of the Meiji restoration. It is against this particular background that the importance of Imanishi's oppostion to Darwinism must be viewed.

Imanishi is still a national hero, as he was in the 1930s. The popular press abounds with interviews and articles by and about him. Typical is the article in the English language magazine *Look Japan* (10 January, 1980) "Kinji Imanishi, Japanese Theorist — as compared to Darwin", in which the three great thinkers of modern times are seen as Darwin, Marx and Imanishi. The interview with Imanishi in the July 1984 issue of *Omni* (Japanese edition only) gives a very clear idea of the high esteem in which he is held.

Asquith[9] confirms my critique of Japanese scientists. According to her "the majority of Japanese scientists simply disagree with Imanishi's popularizations and find his views obscure and untestable. Their silence is hardly surprising". My account was an attempt to draw attention to Imanishi's theories and to set them in their context, and if possible to provoke the Japanese scientific community into some kind of response. Sibatani's[16] initial aim was to bring Imanishi's ideas to the attention of Western scientists. I was the first to respond. I set out in my book[17] to bring to the attention of Japanese scientists the considered opinion of an individual Western scientist on the theories of Imanishi. I believe that both Sibatani and I have succeeded in our avowed intentions.

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● This correspondence is now closed — Editor, *Nature*.

Macrophage regulation of vitamin D_3 metabolites

SIR—In his provocative News and Views summary[1] of some features of the vitamin D system, Ian Dickson discusses the formation of the active 1,25-dihydroxyvitamin D_3 metabolite from 25-hydroxyvitamin D_3 by the enzyme 1-hydroxylase in the kidney. He also refers briefly to the existence of receptors for this metabolite on macrophages and in the thymus. The considerable importance of the latter observations is more obvious when one realises that macrophages can also express the 1-hydroxylase, which had been thought to occur only in the kidney. Moreover, this activity is enhanced dramatically when macrophages are exposed to gamma interferon[2-4].

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Thus the 1,25-dihydroxy metabolite formed by the macrophages can have an autocrine effect on these cells. For instance, in the system we have studied it increases the ability of cultured human monocytes to inhibit the *in vitro* growth of

Mycobacterium tuberculosis[4], possibly explaining the well documented reports that vitamin D therapy cures chronic skin tuberculosis[5]. Because T lymphocytes also contain the receptor, presumably they are also affected by the metabolite, but with what functional significance is not clear[6].

Thus vitamin D_3 metabolites have immunologically relevant effects on the lymphoid system, and their levels within this system are locally regulated by T cells, gamma interferon and macrophages, in a manner which may usually be quite independent of the regulation of systemic levels. When interferon-induced macrophage activation is intense, however, 'overspill' of the 1,25-dihydroxy metabolite can occur, causing sufficient rises in systemic levels to induce hypercalcaemia, as often seen in sarcoidosis[2], and sometimes in tuberculosis[7].

Once again the macrophage proves to be a cell with an amazing range of potential functions.

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Models for the origin of influenza viruses

SIR—Hoyle and Wickramasinghe have recently repeated[1,2] their hypothesis[3] that the Earth is continually bombarded by influenza viruses from comets. Their 'comet' model denies transmission of influenza viruses between individuals, each infection being contracted directly from virus particles falling through the upper atmosphere. The time is past for such qualitative speculations now that the predictions from the comet model can be falsified by quantitative tests.

The predictions concern the minimal-length trees that can be constructed on the basis of nucleic-acid sequences. The largest set of sequences for one strain of influenza virus from the same host are nine haemagglutinin sequences covering the years 1933 to 1980 of the human H1 subtype. The simplest comet model is that the different isolates of H1 influenza are carried on different comets, having developed independently from a common ancestor that existed at some distant time in the past. On this model the sequences would appear generally similar because of their common origin (thus explaining their homology), but the differences between different pairs of taxa would be independent. This is the Big-Bang model[4], and is represented by the star tree[4,5] shown in Fig. 1*a*. If, for example, three viral isolates had adenine at one nucleotide position and the other six had cytosine then the star tree would require three nucleotide changes while the biological model, which assumes that viral strains share common ancestors (Fig. 1*b*), could require one, two or three changes. The expected number of nucleotide changes for a minimal-length tree, given the Big-Bang model, can be calculated (unpublished data). For these nine sequences, the expected number is 163 whereas the observed number is 95. This has a probability of occurring of about 10^{-58}, assuming that the viruses arose independently from a common ancestor. Thus we reject the star tree model.

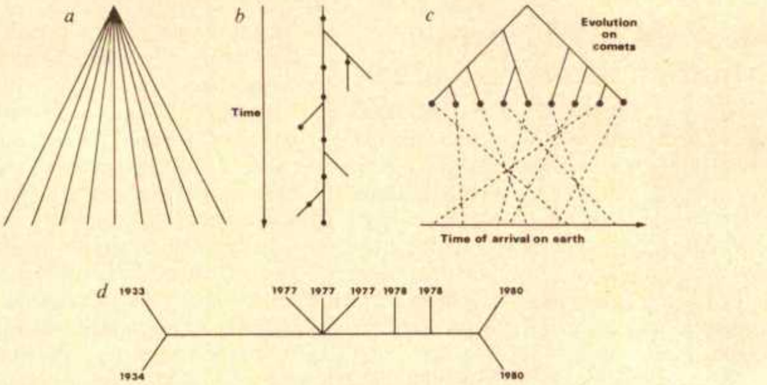

Fig. 1 Trees representing different models. *a*, The Big-Bang or star tree model. *b*, The biological model (for example ref. 7). *c*, Modified comet model (independent arrival). *d*, The minimal tree[8] for the nine viral sequences from the EMBL database[9], edges of zero length are contracted.

The observed number of changes is however consistent with an evolutionary model whether it be terrestrial or extraterrestrial. For example the second version of the comet model (Fig. 1*c*) assumes that viruses on different comets are related through some ancient extraterrestrial evolutionary process but that they arrive independently on earth from different comets. Viruses on two comets may share a more recent ancestor than with those on a third comet but any relationships among the viruses should show no significant correlation with their order of arrival on earth. This leads to the prediction that influenza viruses isolated in consecutive years should not be more closely related than those isolated several years apart. In fact, the viruses on the minimal tree (Fig. 1*d*) are in chronological sequence, consistent with the biological model. There is one chance in 7,560 (=9!/8.6.2) of obtaining this time sequence if the viruses on the minimal tree arrived independently in time. Extending the data set to 12 viral isolates (1933–1982) for which a smaller part (312 nucleotides) of the haemagglutinin gene is sequenced, leads to an even smaller probability. Twelve taxa with independent arrival times, will produce a chronological order as good as that observed (one pair of adjacent isolates interchanged) about once in 1,500,000 trials. Consequently we reject this second comet model.

Models invoking only a single comet do not fit the data either. One such possibility is that of a single comet carrying an evolving virus that periodically infects the Earth as the comet passes it. But the pattern in the arrival of virus isolates, the short times between altered sequences, the lack of a slow mixing compartment in the atmosphere and the lack of randomization of sequences to give a 'quasi-species'[6] lead us to conclude that this single-comet hypothesis cannot explain the evolution of influenza viruses while still retaining the assumption of non-transmission between host individuals.

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Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. They need not arise out of anything published in *Nature*. In any case, priority will be given to letters of less than 500 words and five references. ☐

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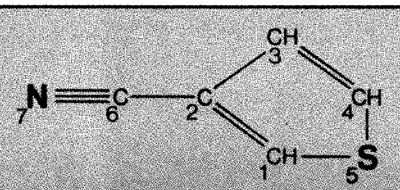

1.	ATOM	C	2211	5	2	0	0	0	0	0	0
2.	ATOM	C	2146	3	6	1	0	0	0	0	0
3.	ATOM	C	2083	4	2	0	0	0	0	0	0
4.	ATOM	C	2148	5	3	0	0	0	0	0	0
5.	ATOM	S	2212	1	4	0	0	0	0	0	0
6.	ATOM	C	2145	7	2	0	0	0	0	0	0
7.	ATOM	N	2144	6	0	0	0	0	0	0	0

Fig. 4. Structure and topological matrix for 3-cyanothiophene.

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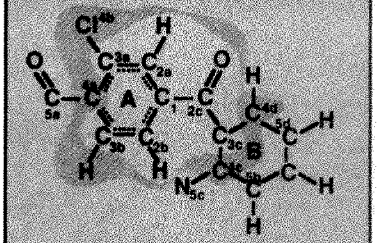

Fig. 6. The four spheres surrounding the focus C-1, with the hierarchical numbering defined by the priority rules given in Table 1.

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A drive with Dr Klein

Jonathan Howard

Natural History of the Major Histocompatibility Complex. By Jan Klein.
Wiley: 1986. Pp.775. $95, £90.75.

JAN KLEIN admits outright that his enormous new book does not easily fall into any of the established categories of textbook, monograph or review. But perhaps the more interesting question for the critic is not what the book is but why it was written. Many and powerful impulsions must have driven Klein to produce a volume of such size and scope, an astonishing labour for one busy man, but the account of them in the preface does not make wholly reassuring reading.

First, Klein rejects what he feels is an aesthetically and morally disreputable view that no single person can come to grips with a whole field; "the philosophy of the assembly line.... I have always thought that if I were to contribute to the construction of a car, I would want to drive it as well". The anxious reader might here wonder whether an assembly line worker is necessarily the best driver. Secondly, there was an impulsion not just to come to grips with the whole field, but actually to *contain* it. With the example of Buffon's 36-volume *Histoire Naturelle*, Klein justifies both his slightly odd title and the attempt to make this account of immunogenetics not merely inclusive in its own field but inclusive of everything. If our first fear was that the drive might be unsafe, our second is that we might well get lost.

Klein's third stated impulsion to write this book is the most unsettling of all: "I also hope to have achieved one further goal: that of discouraging certain lines of research". There are, perhaps, some innocuous readings of this extraordinary declaration, but a glance at Table 7.42, to which Klein refers the reader in this context, makes it clear that they do not apply. There are not many fundamental problems left in immunology, but one that would be on anybody's list is the subject of this table, namely the relationship between T-cell function (that is, help, cytotoxicity and suppression), the specificity of the T-cell receptor for class I or class II MHC molecules, and the expression of the co-receptor molecules, CD4 and CD8 or their homologues. To our other fears, therefore, we must add that the driver seems to have taken an unreasonable dislike to the opinions of most of his passengers.

It must be admitted that these fears are not groundless. Klein's great book, *Biology of the Mouse Histocompatibility-2 Complex*, was subtitled *Principles of Immunogenetics Applied to a Single System*. Ten years later the boot is on the other foot, and it is the MHC whose principles apply to many systems. The MHC is a peculiar object of study. Functionally a part of the mechanism driving the immune response, to which uniquely, as I and Klein both believe, it owes its properties, it now also provides an object lesson in the study of balanced polymorphism, multigene families, ecological and evolutionary genetics, genetic mechanisms such as mutation, gene conversion and recombination, developmental regulation and much more. Klein's instinct that this topic contains something of value for every biologist is surely correct. However the relevance of MHC studies to so many disciplines puts the would-be expositor into a peculiarly difficult position. There is no real 'field' of the MHC for Klein or anyone else to come to grips with. For example, immunology is a highly-structured discipline in which the MHC-specified glycoproteins play a central role, but the genetic complexity at the chromosomal and population levels, which provides much of the MHC's renown, is, in the context of immunology, often a convenience, usually an irrelevance and occasionally a damn nuisance. In other disciplines it is the genetic properties of the MHC that matter, while the immunological activity of this or that product is beside the point. In the case of human tissue typing it is the sheer diversity that matters, with all the impenetrable nomenclature that follows.

Jan Klein — a man of conviction.

With the most extraordinary courage, Klein has taken on the whole thing. Following an approach that he pioneered in his 1982 textbook, *Immunology: The Science of Self–Nonself Discrimination*, he tells us not only everything that he knew before he started work on the book, but also everything that he found out after he got going. As a result, what was already certain to be a mighty production seems almost to have burst the bounds of reason. Where else can you find a detailed account of DNA sequencing by limited chemical cleavage but no mention of dideoxy chain termination? A weird mnemonic for the single-letter amino acid symbols (Tryptophan = W? tWo rings, of course)? A picture of *Lens culinaris* from which lentil lectin is purified? Gametogenesis and sexual morphology in flowers? The life-cycle of bacteriophage lambda? And so on and on. Klein–Diderot more than Klein–Buffon.

One would, perhaps, be better prepared for this torrent of information if Klein were not so dreadfully strict about other matters. For Klein, the MHC is precisely and exclusively "that group of genes coding for molecules that provide the context for the recognition of foreign antigens by T lymphocytes". No complement genes, no steroid hydroxylases, no neuraminidases, none of the things that one might vaguely include in the MHC by virtue of the fact that they are usually all bundled up with Klein's genes on the same chromosome, even if one does not understand why. On the other hand, Klein points out that his definition allows him to include β_2-microglobulin even though it maps to a different chromosome. In the event one need not worry too much about possible exclusions, because, in the encyclopaedic spirit, there is in fact a chapter devoted to them, including among an enormous number of other things a picture of the whole complement system, much of which is nothing to do with the MHC by anyone's definition.

Such a book makes exceptional demands on the index; sadly, that provided is not up to the task. Anyone who wished, for example, to track down the passage on page 137 where Klein describes the evidence that at last convinced him that unidirectional genetic exchange was responsible for much of the peculiar sequence variation in MHC genes will not, surprisingly, find it under either *Gene Conversion* or even *Conversion*, even though at least the latter would seem entirely appropriate.

The sheer prodigality of the information carried in this book, whether in the text, the figures or the wonderful tables which enshrine the published history of every MHC-related topic, make it quite unique. But there is a price to pay, and I do not mean £90.75. This is a book of opinion and judgement as well as fact, and while Klein's views are often balanced and helpful, as in the excellent discussion of the *Mls* locus problem, they are often capricious and sometimes plain offensive, as in the repeated insinuation that his fellow scientists have obtained results in bad faith. When the topic is centrally important to immunology, this high-handed treatment is a serious defect. For instance, the handling of antigen before it is presented to specific lymphocyte receptors is crucially important, and, unlike a lot of material in the book, is strictly relevant to the properties of MHC molecules as well. Unfortunately it is also one of the topics where Klein feels that most immunologists are wrong-headed. That is to say, not only does he feel that he is right, but also that it is a topic which is best presented in such a way that some hypothetical objective reader can appreciate how silly most immunologists are. The trouble with this approach, apart from the unpleasant sensations that it excites in someone who might have inclined to the majority view, is that it fails to prepare the reader for the proper appreciation of work published after Klein's book was completed. It is especially sad in this case. Last year it was shown that membrane class I and class II MHC molecules probably acquire processed antigen from different intracellular pools. If generally true, this finding has tremendous implications for the future development of immunology, especially the immunology of virus infection, as well as making a dramatic and so far unassimilated contribution to cell biology. It is also giving most immunologists a great deal of enjoyment. An authoritative teacher who ridicules an important argument that turns out to be right has a lot to answer for.

But it is always a pleasure to read Jan Klein, even when one disagrees with what he says or disapproves of how he says it. He is a fluent writer with an enviable ability to say clearly what he means. His writing is, in that sense, transparent, so that one seems to see the man himself speaking through the page. He has enormous convictions, and it is difficult not to fall foul of at least some of them. But Klein has enormous courage too, an important virtue and one that demands an equal and often opposite courage in his audience. Do not necessarily believe what Klein says; take the trouble to find out your own point of view for yourself. If it conflicts with Klein, then take issue with him; he may well be wrong. If he forces you to think again about why you believe that something is so, even if you end up with your own conviction strengthened, then he has done something of rare value. □

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Who dunnit down at the gravel pit?

Michael Day

The Piltdown Inquest. By Charles Blinderman. *Prometheus: 1986. Pp.261. $22.95.*

It is a strange facet of humanity that, as a species, we are intensely concerned with transgressions of our own codes of behaviour. Distinguished academics write whodunnits, television programmes are obsessed with crime and its detection, successful art forgers such as Van Meergeren and Tom Keating are folk heroes, and even the Great Train Robbery is part of modern British history. It is not surprising, therefore, that the Piltdown Forgery has retained its place as the most famous scientific fraud of this century. The recovery from a Sussex Pliocene gravel pit in 1908 of a piece of 'human' fossil skull began a series of so-called discoveries that culminated in the reconstruction of Piltdown Man from what turned out to be an orang-utan jaw and other fossilized skull bones. The skull was believed by many to represent an example of the 'missing link' between ape and man that seemed to be needed to give substance to Darwin's view of the origin of species as applied to man.

It was not until 1953 that the fraud was exposed by the use of the modern techniques of the day, including fluorine analysis of bone, analysis of the paint on the specimens and the detection of radioactivity in some hippopotamus teeth in the pit that gave away their provenance. The forgery was exposed in 1953, but not the forger. Over the past 30 years, almost everybody who was in any way connected with the discovery or examination of the remains has been a suspect, and many books and articles have been devoted to proving that one or other famous figure was the perpetrator. Blinderman's book, the latest in the line, is a timely review conducted in the form of a historical inquest; it is well researched, accurate and readable, even if its style is racy on occasion. Blinderman writes with assurance, if not authority, evidently gained from an exhaustive search of the literature and other extensive inquiries.

Following the story of the discovery and the exposure of the forgery, Blinderman offers a profile of the hoaxer; then the ten main suspects are examined one by one and the field narrowed down. The line-up of the culprit (or culprits, for conspiracy theory is not ruled out) includes, alphabetically, Lewis Abbott, William Butterfield, Charles Dawson, Arthur Conan Doyle, John Hewitt, Martin Hinton, Grafton Elliot Smith, W.J. Sollas, P. Teilhard de Chardin and Samuel Woodhead. Clearly some of these are more suspect than others. Foremost amongst them, of course, is Charles Dawson, the local solicitor and scientific savant who 'found' many of the pieces and who is the culprit favoured by J.S. Weiner, one of the prime movers in the uncovering of the hoax. It is true, however, that Weiner was persuaded to name Dawson publicly only when pressed by Lord Zuckerman in 1972 after accusations directed at Grafton Elliot Smith had distressed Smith's family.

Perhaps the only piece that I can add to the jigsaw is that Weiner must have the credit for realizing that the explanation for the strange anatomy and the fluorine results had to be fraud. Weiner told me that after visiting the British Museum and examining the Piltdown remains yet again, he drove home to Oxford one evening. While musing on the day, he realized that the lack of co-planarity in the wear pattern of the Piltdown molars could have been produced only by artificial means. The teeth must have been tampered with after death. As with every conjuror, you fail to understand the trick because you never believe that he would be so mean.

After that flash of insight, the other features fell into place; the broken condyle, the brown paint and the jaw's high nitrogen content. The ape-like anatomy of some parts of the jaw was also explained — simply, it had belonged to an ape.

There is no doubt that the Piltdown forger was knowledgeable, skilful, cunning and fortunate. The gullibility of the scientists at the time seems likely to have been due not to stupidity but to a desire to see evidence for a theory that was so compelling and satisfying. The hoaxer could lead them on from find to find, confirming their best guesses every time and feeding their scientific vanity. We are never so vulnerable as when others are confirming our views.

Not all of the scientists of the day were fooled, however. As early as 1913, the British anatomist David Waterston refused to accept that the jaw and the skull had come from one individual, claiming that X-rays proved that the jaw was that of an ape. In the United States, Gerrit Miller took the same view, as did a number of continental authorities. The doubts faded as the forger/hoaxer salted his mines and

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- Just published in the United States (by Houghton Mifflin) is *The Red Ape: Orang-utans & Human Origins*, by Jeffrey H. Schwartz. The book is tendentious, the author examining the "often unacknowledged biases and preconceptions" of palaeoanthropologists and aiming to show that the closest relatives of *Homo sapiens* are not the African apes but the orang-utan.

Publisher in Britain will be Elm Tree, an imprint of Hamish Hamilton, and the book will be reviewed in a future issue of *Nature*.

more and more material came to light. Was it really a hoax that went too far to be admitted? Was it an attempt to make a fool of a rival? Was it a serious but fraudulent attempt to 'prove' Darwin's theory? The question of motive is just as intriguing as the identity of the forger.

Blinderman's inquiries range widely, even entering the realm of fantasy when Conan Doyle is considered suspect and Sherlock Holmes is brought in to help. Does he succeed? Which of the ten suspects is finally convicted? You'll have to read the book to find out. □

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Energetic exercise

Ian Smart

Energy 2000: A Reference Projection and Alternative Outlooks for the European Community and the World to the Year 2000. By Jean-François Guilmot, David McGlue, Pierre Valette and Christian Waeterloos. *Cambridge University Press: 1986. Pp.261. £25, $44.50.*

GIVEN the circumstances of the past 15 years, it takes courage to publish a projection of energy demand and supply. And only the brave would ever reveal the results of an exercise in formal modelling. What is ostensibly a dry discourse is thus doubly valorous.

Building on research promoted by the European Commission over a ten-year period, what we have are accurately described by the sponsors as "consistent energy projections on a country basis and at the level of the Community as a whole, using throughout the same methodological approach and harmonised energy data and balances" (p. viii). The methods are conventional, entailing the choice of a single socio-economic scenario for the latter-day Community of Ten (that is, excluding Spain and Portugal) and using that scenario to establish a reference projection of energy demand and supply until the end of the century, surrounded by less detailed 'high' and 'low' variants. What is less usual and more stimulating is that aggregated results for the Community have been assembled from a series of standardized and consistent sub-projections of national energy markets.

The result is a workmanlike job. Readers drawn by an interest in modelling will regret that there is little specific information about the algorithms employed. Those looking for a reference work will wish that, in the absence of an index, a detailed catalogue of contents and tables could at least have been included. And no one is likely to cherish the text as light reading. Yet the exercise would still have justified itself had it done no more than coax and cudgel evidence from ten different energy economies into a common analytical frame.

In fact, the study attempts more — but not always so successfully. It necessarily has a good deal to say about energy costs and prices, for example. But the handling of how input costs are translated into consumer prices, or prices related to investment propensity, is too tentative and opaque to be convincing. That may be because the Commission is sensitive to how Community members will feel about a study of this sort. Repeating that the authors' energy projection is not a forecast but only "a plausible and consistent scenario" cannot conceal that it reflects what they take to be the probable evolution of policy as much as of economic circumstance. Indeed, their methods included consulting the views of national governments, and the effect is sometimes apparent. Even in 1985, for instance, few analysts would have wagered on Italy commissioning another ten nuclear power reactors before the end of the century, or Britain building not only Sizewell B by the year 2000 but six more similar units as well.

As it is, the plausibility of what is projected here was largely demolished in 1986 by the collapse of oil prices and the disaster at Chernobyl. Ironically, that is almost to the report's advantage, because it removes the temptation to consider it as anything but a valuable effort to make energy systems analysis in ten different countries more rigorous, transparent and consistent. But the extraordinary history of 1986 also throws into relief the study's most fundamental weakness, which lies in its treatment of uncertainty. Many inputs to the model are properly described as uncertain. The output projections, however, still seem to assume that policy-makers will be confidently rational in choosing an optimal response for the long term. As a result, there is no allowance for how traumatic experiences undermine their confidence, and increase the pressure to reject long-term commitments in favour of more easily mutable short-term palliatives. They, too, are uncertain.

This report is testimony to a brave beginning. As good modelling studies should, it purports to give no answers, but only to help in asking progressively better questions. The European Commission should therefore be encouraged to repeat the exercise. If it does, however, it will need to take account not only of economic uncertainty in energy matters but also of nervous managers and frightened politicians. □

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Star-like qualities

Malcolm G. Smith

Quasar Astronomy. By Daniel W. Weedman. *Cambridge University Press:1986. Pp. 217. £25, $39.50.*

Quasar Astronomy is an excellent observers' research guide to the violent universe of quasars and active galactic nuclei. Daniel Weedman has been observing Seyfert galaxies and quasars at a wide variety of wavelengths, using a wide variety of techniques, for the past 20 years. His teaching credentials in this field are equally impressive. As he reminds us in his preface:

> I already owed a debt to the late Carl Seyfert, who had given me my first encouragement in astronomy when I was a high-school student… this book is a product of my attempts to explain [quasar astronomy] to students in various classes at the University of Texas, Vanderbilt University, University of Minnesota and Pennsylvania State University.

From the outset, Weedman makes it clear that technology has played an essential role in the study of these very distant sources of energy. He writes:

> To be honest… most of the progress [in understanding quasars] should be credited to the engineers and physicists who have developed tools that allow our wide-ranging probes into the mysteries of quasars.

That point of view may be a little extreme, and could be contested by a few of the eminent theoreticians who have worked in this field, but the quotation serves to illustrate some of the flavour of Weedman's approach.

This is not a difficult book. Weedman's account is basic and straightforward, and is illustrated with simple worked examples. He takes the view that the complications stemming from the maze of unit conversions and cosmological formulae are important enough that most students of the subject would benefit from having the details explained and set out explicitly. He remembers that most of us took a number of years to become reasonably comfortable with all the practical corrections and conversions — and that most of us worked through many of the derivations essentially from scratch, and then kept the results in a notebook to save having to do them all again!

Weedman's treatment of the physics of quasars is highly simplified. Yet it shows the insight that his deceptively simple observational approach to research has brought to this field, and the book will be of great value to those embarking on the study of quasar astronomy. □

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Changing physics

Cyril Domb

James Clerk Maxwell and the Theory of the Electromagnetic Field. By John Hendry. *Adam Hilger: 1986. Pp.305. £30.**
Maxwell on Molecules and Gases. Edited by Elizabeth Garber, Stephen G. Brush and C.W. F. Everitt. *MIT Press: 1986. Pp.565. $55, £54.95.*

WHEN the Cavendish chair of physics was established in 1871, it was offered first to Sir William Thomson (later Lord Kelvin) and then to Helmholtz. Only when both of these had declined was James Clerk Maxwell approached. Several years had passed since the publication of Maxwell's classic papers on the electromagnetic field, but their significance was far from being appreciated. As late as 1884 Thomson wrote in the preface to his Baltimore Lectures that "the so-called electromagnetic theory of light has not helped us hitherto". Maxwell's contemporaries regarded him as a top scientist, but not as one of the great innovators of history.

The first decades of this century saw the development and exploitation of radio waves, and the formulation of the special theory of relativity and of Planck's quantum hypothesis, in all of which Maxwell's electromagnetic theory played a central role. The physicist of the twentieth century has no doubts that Maxwell deserves to rank with Newton and Einstein. In a celebration of the centenary of Maxwell's birth in 1931, Einstein described the change in conceptions of physical reality after Maxwell as "the most fruitful that physics has experienced since the time of Newton". In addition to his epoch-making ideas on electromagnetic theory, Maxwell made outstanding contributions to statistical mechanics, kinetic theory, colour vision and a host of other topics, and all of this in a short life-span of 48 years. But although Newton and Einstein are household names, the average man in the street has not heard of Maxwell.

During the past few years efforts have been made to put Maxwell on the map where he belongs. The excellent short biography by Everitt in 1974 contained interesting new material (Maxwell died in 1879 and it was still possible to make use of living testimony). More recently, the centenary of Maxwell's death stimulated new biographies by Tolstoy in 1981 and Goldman in 1983.

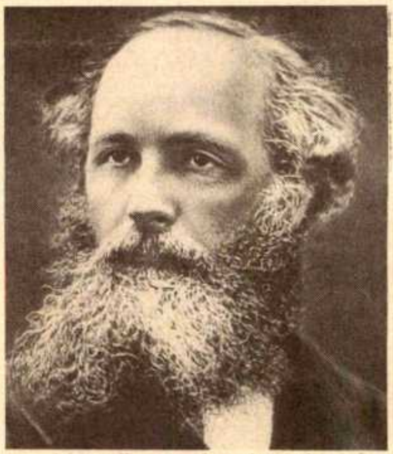
The books under review are welcome additions to the literature on Maxwell, and are designed for a more professional audience of historians and philosophers of science. They deal with different aspects of Maxwell's work, Hendry being concerned with electromagnetic theory, and Garber and her colleagues with statistical physics and kinetic theory. The approaches also differ. Hendry has not made much attempt to unearth new archival material, and has relied on what is already available in the literature, whereas Garber *et al.* reproduce Maxwell's publications and letters in a systematic manner with a learned commentary, one of their aims being to make widely available material which has previously been known only to specialists.

Hendry starts with a comprehensive account of the historical development of electricity, magnetism and electromagnetism at the beginning of the nineteenth century, tracing the contributions of Oersted, Ampère, Faraday and Thomson. This sets the stage for Maxwell's entry. Maxwell was deeply influenced by Faraday's idea of a field filled by lines of force, and his initial aim was to give mathematical expression to this concept. In fact his preliminary paper, written in 1855 while he was still a student at Cambridge, was entitled "On Faraday's Lines of Force". The first two of his classic papers on the electromagnetic field, published in 1861 and 1862 under the title "On Physical Lines of Force", made extensive use of molecular vortices to explain lines of force and electric currents; in the second paper he introduced a mechanical analogue, and this led him to postulate a displacement current. The mathematical consequence of this postulate was the existence of electromagnetic waves with a velocity determined by electric and magnetic quantities. When numerical values were inserted the velocity turned out to be close to that of light. In his final paper, "A Dynamical Theory of the Electromagnetic Field", published in 1865, the superstructure of molecular vortices and mechanical analogues was swept away, and the equations were put forward in their superb symmetric form.

Hendry supports the view of T.S. Kuhn that discovery is rarely a simple event and that it should be treated as a process. He emphasizes that Maxwell's great contribution was the understanding he provided of the nature of electromagnetism. The identity of the velocity of electromagnetic and luminiferous waves was just one point in an elegant and sophisticated structure. Others (including the distinguished mathematician B. Riemann) had suggested this one point, but had been unable to provide an acceptable physical framework.

A good deal of space is devoted to an analysis of the difference between the mechanistic and dynamistic approaches in the history and philosophy of science. I did not find the discussion very relevant, but this may be due to my training as a physicist. One feature caused me some irritation. An important advantage of the system of referencing by dates is the historical perspective gained when one reads the text. This is lost if the reference is not to the original publication. A reference to J.C. Maxwell (1982) is bizarre; it is much more appropriate to write J.C. Maxwell (1865) and in the reference list add 'reprinted 1982...'.

In the second volume under review, the published papers and manuscript writings are handled in a highly professional manner. Both the preface and the introductory article entitled "Kinetic Theory and the Properties of Gases: Maxwell's Work in the 19th Century Context" are useful and informative. Notes on the archival material save the reader from puzzling over unfamiliar phrases in the letters and postcards.



Cavendish Laboratory

Maxwell — epoch-making ideas.

The published papers are drawn largely from the two volumes of Maxwell's collected works edited by W.D. Niven (reprinted by Dover in 1965, though now alas again out of print). But some material which slipped through Niven's net is included here, for example an exchange of letters in *Nature* with A.J. Mott on "Atoms and Ether", and an *Encyclopaedia Britannica* article on "Physical Sciences" published posthumously.

A major source of letters and articles is the classic biography of Maxwell by Campbell and Garnett, published within a few years of Maxwell's death and happily available again as a Johnson reprint. But the most novel features of the book are the letters and manuscripts reproduced from private collections, most of them coming from the Maxwell collection in the Cambridge University Library. Other letters have already appeared in print but are scattered through, for example, biographies of Stokes and Kelvin.

Both volumes provide ample evidence of the care which Maxwell devoted to every statement he made; of his coherent and logical thinking; and of his magnificent command of the English language and unique personal style. ☐

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Editor: Y. A. Kozlovsky, Moscow
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1986. 190 figures. XIII, 558 pages. (Exploration of the Deep Continental Crust).
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The superdeep well on the Kola Peninsula near Murmansk, begun over 15 years ago, has reached a depth of 12 km and is thereby the deepest well ever drilled. The vast amount of information on the structure of the continental crust which has been gained by Soviet scientists is now made available in English translation to scientists worldwide.

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Editors: H. D. I. Abarbanel, W. R. Young, University of California at La Jolla

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Programming a highly parallel computer

W. Daniel Hillis and Joshua Barnes

Parallel computers potentially offer rates of computation far above those of conventional machines but using them efficiently requires understanding of their capabilities and limitations. Algorithms that simulate the N*-body problem on an existing parallel computer with 65,536 processors provide an illustration*

A NEW type of computing engine, the parallel computer, offers opportunities to perform scientific computation that have previously been impossible. Unlike conventional computers that perform one operation at a time, parallel computers can perform tens of thousands of operations simultaneously, making them very much faster. But high performance cannot be achieved for all algorithms. Just as the electron microscope offers a far higher magnification than the optical microscope, without replacing it, the parallel computer offers much greater rates of computation for certain problems, but it is not a direct replacement for conventional sequential machines. Here we shall review some of the capabilities of a 'fine-grained' parallel computer, one that contains a large number of computational elements and is particularly suited for the study of systems containing a large number of interacting elements.

The algorithms described are 'data parallel' rather than 'control parallel' in that they can perform simultaneously atomic operations on large sets of data. For example, adding two large vectors containing many components is an atomic operation on a parallel computer in the same sense that adding two numbers is an atomic operation on a conventional computer. This type of data parallelism may be contrasted to control parallelism, where concurrency is achieved by coordinating the activities of many programs simultaneously. The data-parallel style of programming is a natural one when tackling computations involving large numbers of similar elements, as are often found in scientific problems. In the example that we discuss, the discrete elements are the positions and velocities of individual stars and the gravitational forces between them. The computational problem is to simulate the motion of the stars over time.

In reality, all stars move simultaneously, so it is natural to model the system by an algorithm in which all elements of the data structure are computed simultaneously. In the execution of the algorithm, an individual computer processor is attached to each element of data, in this case the position or velocity of a single star or the force between two stars. The algorithm is executed in all the processors at once, and hence for all the stars at once. Each step of the algorithm operates on a large set of data elements. In the algorithms we describe, time in the execution of the algorithm is directly analogous to time in the modelled system, although this is not a necessary feature of a data-parallel algorithm.

Parallel architecture

The structure of conventional algorithms reflects the word-at-a-time structure of conventional computers. In the same way, data parallel algorithms reflect the structure of those parallel computers that contain tens of thousands of relatively simple processors. Such machines are referred to as 'fine-grained' parallel computers, in contrast to 'coarse-grained' computers, which contain up to a few hundred relatively conventional processors. As an example of a fine-grained parallel computer designed to be programmed in the data parallel style, we shall describe the system called the Connection Machine[1] (Thinking Machines Corporation) a commercial parallel machine containing 65,536 processors, each with its own memory. Other examples of fine-grained computers are the Massively Parallel Processor[2] (Goodyear Aerospace) and the Distributed Array Processor[3] (ICL).

The Connection Machine has three major components: the processor/memory cells, a conventional front-end computer and a communication network that allows the processor/memory cells to exchange information in arbitrary patterns. The front-end computer is a standard sequential scientific computer (such as DEC's VAX computer). The user programs the computer from the front end, using simple extensions of conventional high-level language, with conventional control structures.

The Connection Machine processor/memory cells connect to the front-end computer as if they were a large conventional memory. In its simplest mode of operation, the processor/memory cells of the system can act simply as memory, supporting read-write operations of the front-end machine. Data stored in this section of the memory can be operated upon in the standard word-at-a-time manner, but it is also possible to perform operations on all of the data at once. For example, all stars can simultaneously update their positions according to their velocities. The program sequence that determines the algorithm is stored once, in the front end. Connection Machine hardware broadcasts each command to all processors, so that each can perform a local operation on its own unique data. The time required to perform elementary operations depends on the size of the data field. For example, operating in parallel, a Connection Machine system is able to do 8×10^9 8-bit additions, or 1×10^9 64-bit additions, per second.

Many important operations require communications between processors. Communication is necessary, for example, to compute the gravitational interaction of the stars. Fine-grained computer systems differ in the type of hardware mechanisms that are provided for in such communication. In the Distributed Array Processor and Massively Parallel Processor systems, the processors are wired in a two-dimensional grid. In the Connection Machine system the processors are interconnected by a switching network that allows any processor to communicate with any other. This operation, which allows processors to move data to and from one another's memories, is similar to 'indirect addressing' on a conventional machine. Using this mechanism, the system is able to move $\sim 2.5 \times 10^8$ 32-bit words of data per second.

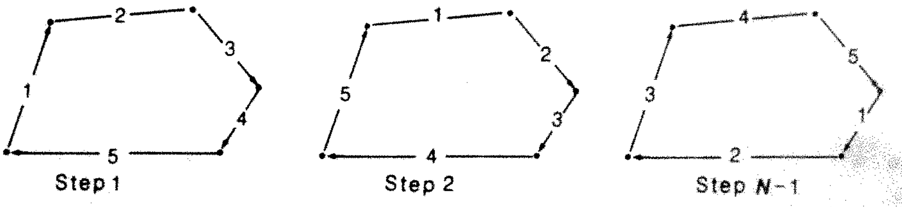

Fig. 1 'Digital Orrery' algorithm shown schematically for $N = 5$. Messages with positions and masses are passed around the ring, the data from each particle visiting all $N - 1$ other particles.

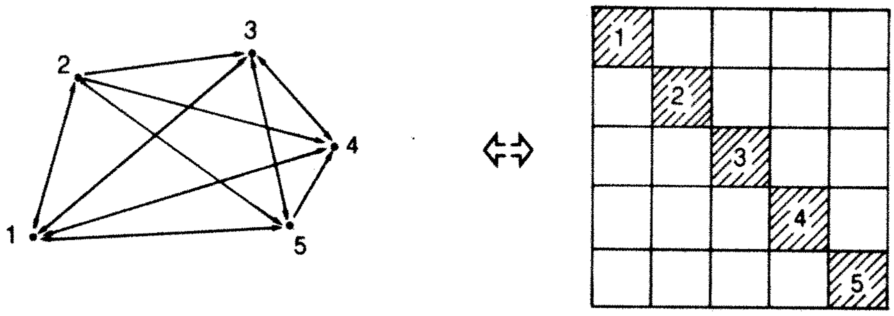

Fig. 2 'Simultaneous' force calculation algorithm for $N = 5$. All $N(N-1)$ interactions are computed in parallel (open squares on checkerboard).

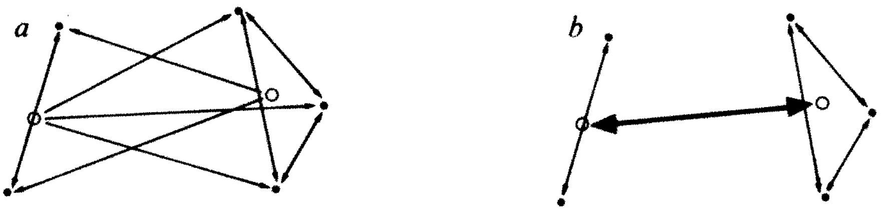

Fig. 3 'Hierarchial' force calculation algorithms. In *a*, particles (solid circles) receive messages directly from other particles and from centroids (open circles) representing distant groups of particles. In *b*, a variation in which long-range interactions computed directly between centroids (thick arrows) are shown, as well as communication between particles (thin arrows).

The overall flow of information during these communication cycles may be divided loosely into fixed pattern and general pattern communications. Fixed pattern communications come up regularly in scientific computations; for example, filtering a two-dimensional image may require a fixed two-dimensional pattern of communication, or simulating the flow of a fluid by fixed-grid numerical methods may require a fixed three-dimensional pattern. Such regular motion of data between cells representing an n-dimensional grid is an operation supported directly by most fine-grained machines. These fixed pattern communications take place in approximately the same time as required for addition.

Another common fixed pattern of communications is that of a binary tree. An efficient method, for example, for computing the total kinetic energy of all of the stars in the system, is first to add the energies in each of 32,768 pairs of stars, and then again add each of 16,384 pairs of pairs, and so on. Each of these operations require a single step in a parallel machine, so 15 steps are required to add the total energy of all 65,536 (2^{15+1}) stars. The communication structure of such a computation is a 15-deep binary tree. Computations of this type are also supported directly by the Connection Machine's communication network. A related communications structure is the 'perfect shuffle' pattern of the fast Fourier transform. This is also supported by the hardware. In the fixed-pattern communication the pattern of data motion is determined by the algorithm, rather than by the data.

In many scientific calculations, the pattern of communications is data-dependent. For example, in efficient algorithms for inverting large sparse matrices, the pattern of communication will depend upon the pattern of non-zero entries in the matrix. Equivalently, in solving a loosely-coupled system of differential equations using numerical methods, the communications patterns will depend on which variables interact. Another example occurs in simulating the flow of a fluid with a non-uniform grid so that portions with more detailed structure are simulated to a higher degree of accuracy. Each of these examples requires motion of data between cells in an irregular pattern that depends on the particular structure of the problem. This type of data-dependent communication is supported on the Connection Machine through the use of indirect addressing. If one processor stores the address of another, then the first processor is able to access the memory of the other through the communications network. An arbitrary pattern of connections between the processors can be created by storing the appropriate pattern of addresses.

In some scientific applications the natural communications pattern changes during the course of the computation. For example, in calculating the flow of fluid it may be best to use an adaptive grid method where more processing is assigned to an area of space whenever turbulence occurs. The adaptive grid 'patches in' a finer mesh structure only where, and when, it is needed. The Connection Machine supports this dynamic storage allocation. Assume that some processors (Subset F), are kept free for this purpose. Other processors (Subset S) are identified as needing assistance at a given moment. Pointers to one or more free processors are set up in each processor in subset S, so that processors in S can use the communications router to distribute portions of the work to the newly allocated processors. This type of allocation of free processors is supported as a primitive operation.

The *N*-body problem

As an example of a scientific computing problem which may take advantage of a parallel computer, we consider one of the oldest problems in mathematical physics, the evolution of a system of N self-gravitating bodies. One idealization of this problem is expressed in the following equations of motion:

$$\frac{d^2 \mathbf{r}_i}{dt^2} = \sum_{j \neq i} \frac{G m_j (\mathbf{r}_j - \mathbf{r}_i)}{|\mathbf{r}_j - \mathbf{r}_i|^3} \quad i = 1,\ldots,N \qquad (1)$$

where m_i and $\mathbf{r}_i$ are the mass and position respectively of body i and G is the gravitational constant. The formulation and solution of this problem for a system of $N = 2$ bodies was one of the first major triumphs of newtonian mechanics. For $N \geq 3$ no general solution is known. Over the past 300 years considerable progress has been made in developing analytic approximations for special cases, including the Solar System. For large-N systems such as star clusters ($N \simeq 10^6$ stars) and galaxies ($N \simeq 10^{11}$), only a handful of equilibrium solutions are known, and many questions about the existence, stability, dynamical evolution, and interactions of such systems pose intractable mathematical problems.

With the introduction and steady improvement of digital computers, numerical simulations of so-called 'N-body models' governed by equation (1) have become an increasingly important tool for studying the dynamics of self-gravitating large-N systems. (For many purposes in the study of large-N systems, an approximation of the exact r^{-2} force law in (1) may be acceptable. One standard approximation, which has the advantage of removing the singularity when $r = 0$, is to replace the denominator in (1) with $(|\mathbf{r}_j - \mathbf{r}_i|^3 + \epsilon^2)^{3/2}$, where ϵ is a small 'softening' parameter with dimensions of length.) Such simulations tend to be computation-intensive, because evaluating of the summation in (1) requires $O(N)$ operations to obtain the force on one particle. A naive N-body code therefore takes $O(N^2)$operations per timestep. Alternatively, the gravitational potential may be expanded in a suitably chosen set of basic functions. Algorithms of this kind require significantly fewer operations.

However, potential-expansion codes often lack generality and suffer from limitations on the range of length-scales which may be studied.

Conventional N-body algorithms that explicitly loop over all bodies $j = 1,\ldots,N$ to evaluate the force on body i may be adapted to use a Connection Machine with relatively trivial modifications. One processor is used for each body, so that the forces on body i due to each body $j \neq i$ are computed simultaneously in processors $1,\ldots,i-1, i+1,\ldots,N$. The total force on body i is then obtained by adding up these forces, an operation which may be accomplished in time $O(\log N)$ using a fixed binary-tree communication pattern.

Parallel algorithms

More interesting are N-body algorithms designed for parallel machines. These algorithms can offer significantly higher levels of performance; they also reveal much more of the methodology of parallel computing. Here three examples are presented. These algorithms are described in terms of systems of abstract machines communicating with each other in various patterns, all of which may be efficiently implemented on a Connection Machine.

First, we consider the 'Digital Orrery' algorithm[4], in which one processor is assigned to each particle, and all N particles are linked together in a ring, as in Fig. 1. Positions and masses of particles are passed around the ring. All processors execute the same basic step: forward a message to the next processor on the ring, and compute a two-particle interaction using the message received in turn. A complete force calculation requires $N - 1$ such steps, after which $N(N-1)$ interactions have been evaluated. Velocities and positions may then be updated using any one of a number of possible integrators (for example, leapfrog, Runge–Kutta, predictor-corrector), and the whole process is then repeated. The main advantage of this approach is simplicity; the main disadvantage is the limited scope for improvement on the basic algorithm. The timestep must be short enough to resolve the shortest timescale of dynamical interest, which may be very small compared to the overall evolution time of the system. With a high-order integrator, the numerical error (usually measured by the change in the calculated binding energy, which should be conserved) may then be made quite small; however this leads to only a small increase in the timestep for a fixed level of permissible error.

A simple code of this kind with a time-centred leapfrog integrator has been tested on a Connection Machine. For $N \simeq 2{,}000$ particles, a timestep takes ~ 1 min, with each processor computing ~ 30 newtonian two-body interactions per second.

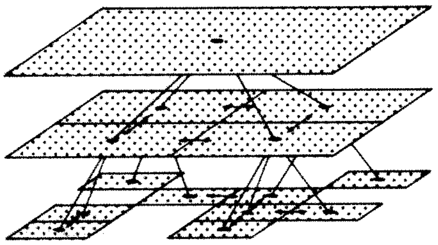

Fig. 4 The two-dimensional analogue of the 'Eulerian' tree structure used in the hierarchical algorithms, showing how the root cell (top) is recursively divided into smaller cells. Here, $N = 9$.

The timing provides the basis for most of the estimates made here. A typical calculation spanning a few dynamical times will require ~ 500 timesteps, so the practical limit is $N \simeq 4{,}000-8{,}000$ if interesting calculations are to run in ~ 1 day. Such a calculation would only use a fraction of the 65,536 processors on a full-sized Connection Machine. To use a 65,536-processor machine efficiently, we can run 8–16 such calculations at the same time, exploiting parallelism at the level of experiments.

A second algorithm is in the 'Simultaneous Force Calculation' algorithm. Instead of assigning one processor per particle, $O(N^2)$ processors may be used to compute all two-particle interactions simultaneously. In the simplest version, sketched in Fig. 2, processors are laid out in an $N \times N$ grid, with those on the diagonal representing particles. At the beginning of a force calculation, each particle processor sends its current position to all other processors in the same row and column. Each off-diagonal processor then computes a single two-body interaction. The resulting forces are summed up and used to advance particle coordinates with a standard numerical integrator.

A non-local communication pattern is required to implement this algorithm. Both the broadcast and the subsequent force summation would take $O(N)$ steps if implemented naively by the one-dimensional communications pattern as used in the Orrery algorithm. These tasks can be accomplished in $O(\log N)$ steps with fixed binary-tree communications patterns. The simultaneous force calculation algorithm can attain very high speeds when applied to relatively small-N systems. For example, a system of $N = 256$ particles would run at a rate of ~ 16 timesteps per second on a Connection Machine; a typical calculation might require half a minute. Larger systems can be run by using each physical processor to simulate several 'virtual' processors, an option supported transparently by the Connection Machine. The limit with existing hardware is probably $N \simeq 1{,}000$. A fast code of this kind can be very useful in exploring the parameter space of interest before performing large-scale calculations.

Third, there are 'Hierarchical Force Calculation' algorithms, in which the total force on a particle may be approximated by summing over a subset of $O(\log N)$ interactions instead of the full $N - 1$ (ref.5.) Two possible versions of this approach are illustrated in Fig. 3. On the left, we show a scheme in which each particle receives messages from (1) particles which lie nearby, and (2) centroids that represent many particles grouped together using a centre-of-mass approximation. As messages come in they are used to calculate accelerations, which are added to a running sum. On the right, we show an approach in which long-range two-body interactions are computed directly between centroids, and local interactions take place between particles. The total acceleration on a particle then includes the partial terms collected by centroids that claim that particle as a member.

Eulerian tree

The flow of messages required to implement these hierarchical algorithms may be organized on a tree structure, in which leaves represent particles and interior nodes represent the centroids. On a Connection Machine, this tree structure employs one processor for each node. Following Barnes and Hut[5], an 'Eulerian' tree that divides space into a nested hierarchy of cubical cells (Fig. 4) is constructed, starting with the particles and grouping nodes together into larger cells. The tree is regenerated after each integration timestep, ensuring that it accurately reflects the particle positions. The regular structure of the tree permits the message flow patterns to be specified in purely geometrical terms, greatly simplifying the design of these algorithms. Messages move through the tree in two directions: laterally between nodes on the same level (through auxiliary cross-links), or vertically from interior nodes toward descendent cells and particles. Each node broadcasts its mass and centre-of-mass position laterally to a limited neighbourhood of nearby nodes. On receiving this information, a cell may forward it vertically to be consumed by descendant particles; this implements the version shown in Fig. 3*a*. Alternatively, messages received by a cell from other nodes on the same level may be consumed locally to obtain a partial acceleration, which is subsequently added to partial accelerations from other levels of the tree to obtain the total force on the particles; this implements the scheme shown in Fig. 3*b*.

The speed of these algorithms depends on the choice of neighbourhood, which may be varied to trade computational throughput for accuracy. With a preliminary implementation of the algorithm shown in Fig. 3*a*, a force calculation for $N = 32{,}768$ particles takes ~ 5 min; as expected, this time scales only as $O(\log N)$ (ref. 7). Interesting calculations may be run in a few hours to a day. The variation shown in Fig. 3*b* is likely to be an order of magnitude faster, although the additional approximation involved in this scheme must be carefully studied before a conclusive comparison can be made. With virtual processors, systems of up to $N = 2^{18}$ particles could be simulated with existing hardware. For an application requiring a guaranteed level of accuracy, a parallel version of an adaptive multipole algorithm could be used[6]. The communications requirements would be similar.

Applications

The scientific applications of these algorithms are extensive. Problems such as collisions between disk galaxies require a very large number of particles (to model the disk) and high spatial resolution (to handle a wide range of scales). Very few self-consistent collisions between disk+halo galaxies have been attempted. Other possible applications include studies of dynamical stability and violent relaxation.

The self-gravitating body problem fits well on a fine-grained parallel computer because, like so many other computations in science, it is inherently parallel. Since the physical world operates in parallel, parallel algorithms are often closer to reality than their serial counterparts. It may be that parallel computers will be easier to program than the sequential machines. Parallel computers will almost certainly become more powerful and more widely accessible in the near future, so we will soon have the opportunity to learn.

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REVIEW ARTICLE

Water on Mars

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Estimates of the amount of water outgassed from Mars, based on the composition of the atmosphere, range from 6 to 160 m, as compared with 3 km for the Earth. In contrast, large flood features, valley networks, and several indicators of ground ice suggest that at least 500 m of water have outgassed. The two sets of estimates may be reconciled if early in its history, Mars lost part of its atmosphere by impact erosion and hydrodynamic escape.

THE amount of water outgassed from Mars has been a controversial issue ever since 1972 when the Mariner 9 spacecraft returned pictures of large channels incised into the planet's surface. The controversy stems in part from an apparent discrepancy between evidence from the surface which suggests that abundant water has outgassed and evidence from the composition of the atmosphere which suggests that little water has outgassed. A reconciliation appears underway in favour of the higher estimates. The reconciliation has been facilitated by a decade of analysis of Viking data, by recognition that the SNC meteorites probably come from Mars and by identification of several processes whereby a planet may lose part of its early atmosphere. The amount of water at the surface is of considerable geological, climatological and biological interest. Water plays a crucial role in many geological processes, including both deep-seated magmatic processes and surficial changes such as weathering and erosion. Because most of the water is believed to have outgassed early on, the near-surface inventory also provides clues about the accretion of the planet, the thermal and fractionation history of the interior, and the formation of the atmosphere. The amount of water also has climatological implications which in turn affect estimates of the probability that life could have started on the planet. There may even be practical implications, for water is a potential resource that future explorers could use for fuel and sustenance. This article summarizes the evidence on which the current inventory of water is estimated, suggests where the water may now be, and how its distribution may have changed with time.

Assessment of the amount of water present is severely impeded by the stability relations of water under current climatic conditions. With a mean atmospheric pressure (due to CO_2) of 6 mbar and mean diurnal temperatures that range from 150 K at the winter pole to 220 K at low latitudes, liquid water is unstable everywhere on the surface, ice is stable only at the poles[1,2], and a thick permafrost zone ranges in thickness from several hundred metres at the equator to kilometres under the poles[3-5]. The only place on the surface where water ice has been unambiguously identified is at the north pole where evaporation of the seasonal CO_2 cap in summer exposes the water ice[6,7]. Ground ice is stable at depths greater than several centimetres at latitudes above about 40° (refs 1, 2). At lower latitudes, however, ground ice is unstable at all depths, and will sublime into the atmosphere at rates dependent on the porosity of the overlying materials. Although the atmospheric pressure may change cyclically because of obliquity variations[8-10], such changes are unlikely to be large enough to alter significantly the stability relations just described[11]. As a consequence, the near-surface materials at low latitudes should, over geological time, have lost any unbound water down to depths of tens to hundreds of metres[12,13]. Such losses could, however, be partly replenished by outgassing of water from greater depths or by subsurface migration from higher latitudes[14].

The column abundance in the atmosphere ranges from less than 5 precipitable micrometres (pr. μm) in the winter hemisphere to over 100 pr. μm over the northern summer pole[2]. The average content is about 10 pr. μm. Although these amounts are very small, the lower 1 km of the atmosphere is close to saturation for night temperatures[15]. The seasonal variations in water content of the atmosphere are due to exchange with the regolith, trapping and release of water in the seasonal CO_2 cap, and loss from the permanent northern water-ice cap[15].

Climatic factors thus prevent liquid water at the surface, allow

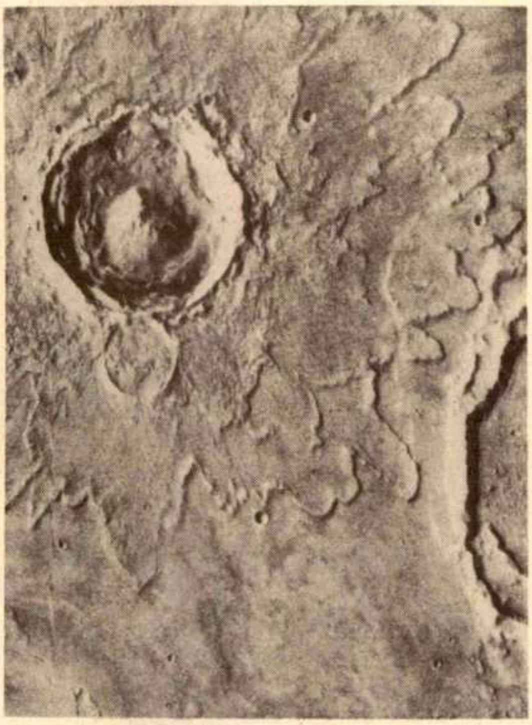

Fig. 1 The 18-km diameter crater Yuty (22° N, 34° W). The ejecta are arrayed in discrete lobes that appear to have flowed outward away from the crater. The lobes are in sharp contrast to the disorganized blocky debris, typical of lunar craters. The fluidity of the debris has been ascribed to entrained water. Such patterns are amost universal around Martian craters larger than 3-5 km possibly because these craters penetrate the thick permafrost.

ice at the surface only at the poles, and result in only minute amounts of water in the atmosphere. If the planet has outgassed large amounts of water, most must be hidden from view either as ground ice or ground water. As only the surface and atmosphere are accessible to direct measurement, the water inventory must be assessed indirectly. This has been done in three basic ways, from geological evidence, from the composition of the atmosphere, and from models of planet formation.

Early views

Our perception of the amount of water at the Mars surface has ranged widely over the last three decades. During the 1950s the atmospheric pressure at the surface was believed to be 100–250 mbar, and due largely to nitrogen. Abundant water was assumed present, and it was thought possible that seasonal changes in albedo and colour could be due to biological activity. This view of Mars was shattered in the early 1960s by measurements of surface temperatures that were inconsistent with any significant greenhouse warming[16], by spectroscopic measurements which showed that the surface pressure was close to 4 mbar (ref. 17) and that only about 15 pr. μm of water was present in the atmosphere[18], and by pictures from the Mariner 4 spacecraft that showed a lifeless cratered surface, much like that of the Moon[19]. This new view of Mars as an inert body, devoid of any evidence that liquid water had been on its surface, appeared confirmed by Mariners 6 and 7. But our perception changed again when, in 1972, the Mariner 9 spacecraft sent back numerous pictures of large channels, which appeared to be clear evidence that large amounts of liquid water had flowed over the surface[20,21]. By this time the stability relations of water, outlined above had been well established[1], so the channels were viewed as evidence for major climate changes in the past[22]. Although the geological evidence suggested that Mars had outgassed significant amounts of water, no quantitative estimates were made of the amount at that time. The supposition that Mars could have outgassed large amounts of water was consistent with most current models for the formation of the planets which predicted that at least the initial inventory of volatiles should increase with increasing heliocentric distance. This appeared true whether accretion was homogeneous[23] or inhomogeneous[24], and whether the density differences between the terrestrial planets are due to fractionation of silicate and metal particles[25] or the oxidation state of the accreted materials[26]. Just before the Viking mission, it was argued from geochemical evidence that Mars had outgassed as much water as the Earth[27,29], but these arguments were based on a substantial overestimate of the abundance of argon in the atmosphere.

Estimates from atmospheric composition

Measurement of the composition of the Martian atmosphere[30] by the Viking landers provided a means of rigorously estimating the amount of water outgassed. Estimates were made in two ways, from the isotope composition of nitrogen and oxygen in the atmosphere and from its noble gas content. From the lack of fractionation of oxygen isotopes McElroy *et al.*[31] showed that the atmosphere must exchange oxygen with some surface reservoir. Assuming the reservoir is near-surface water, they estimated that it must contain at least 13 m of water averaged over the planet; otherwise they would have observed ^{18}O enrichment in the atmosphere. They also assessed the outgassed water from the enrichment of the atmosphere in ^{15}N. The enrichment occurs because ^{14}N is preferentially lost from the upper atmosphere as a result of photodissociation. Reconstruction of the initial inventory of nitrogen is uncertain. It depends on the escape efficiency of nitrogen, isotope fractionation in the upper atmosphere, and the rate of fixation of nitrogen in the surface as nitrates and nitrites, which are all poorly known, but the best estimate of McElroy and co-workers is that the initial surface pressure of N_2 was 20 mbar as compared with the present 0.15 mbar. This implies 120 m of water were outgassed if the H_2O/N ratio was initially the same as on Earth.

Anders, Owen and Morgan[32,33] used the noble gas content of the atmosphere to estimate the outgassed water. The bulk potassium content of the planet was estimated to be between 62–100 p.p.m. from measurements of the radioactivity of surface rocks[34], and from constraints on internal heat production implied by thermal models of the interior[35]. From the bulk potassium content, the amount of ^{40}Ar produced over the history of the planet was calculated, and this was compared with that in the atmosphere to derive an estimate of the fraction of ^{40}Ar released to the atmosphere. This release-factor was estimated to range from 0.067 to 0.099. Since ^{36}Ar was incorporated into the planet during accretion, its release-factor must be at least as large as that of ^{40}Ar. If we assume that all the ^{36}Ar released is still present in the atmosphere, then upper and lower limits can be placed on the global inventory of ^{36}Ar from the present content of the atmosphere. Even the upper limit on the global inventory, which assumes equal outgassing of ^{36}Ar and ^{40}Ar, was estimated as 17 times less than that of the Earth. Anders, Owen and Morgan concluded, therefore, that Mars is considerably less rich in volatiles than the Earth. The global water inventory was estimated by assuming that all volatiles are present in the same proportions as in C3V chondrites. Finally, the amount released to the surface, estimated at 9.4 m averaged over the whole planet, was determined by narrowing further the release-factor by comparisons with ^{129}Xe and by arguing that the release-factor of nitrogen should be no greater on Mars than on the Earth. Rasool and LeSeargeant[36], by similar reasoning, but using volatile ratios in ordinary rather than C3V chondrites, estimated that 6 m had been released. For comparison, the Earth is estimated to have outgassed 3 km of water[24]. The abundances derived from the noble gases are thus very low, contradicting previous estimates of Earth-like abundances, and implying that both the surface and the planet as a whole are very volatile poor.

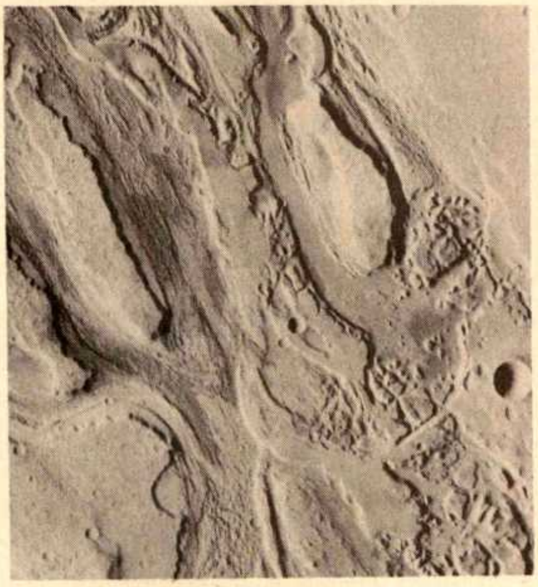

Fig. 2 A section of the outflow channel Mangala Vallis, believed to have been formed by large floods. Branching channels are incised into the surface to depths of several hundred metres. Individual branches are tens of metres across and the entire scene is 100 km across. The large volume of material eroded to form the channels suggests large volumes of water have flowed through them.

Subsequent discovery that the $^{36}Ar/^{12}C$ ratio is 20-200 times larger in the Venus atmosphere than in the volatiles outgassed from the Earth led Pollack and Black[37] to question that the $^{36}Ar/^{12}C$ ratio can be assumed constant in all the terrestrial planets, a basic assumption in the estimation of water on Mars from noble gases in the atmosphere. To explain the Venus results Pollack and Black suggested that different mechanisms controlled incorporation of the noble and non-noble gases into the planets during accretion. Noble gases were mostly adsorbed on grains, whereas other volatile elements were mostly chemically bound within the grains. They suggested that while the planets were accreting, the solar nebula was essentially isothermal with the nebular pressure decreasing outwards. As adsorption depended on the local pressures, Venus accreted from grains with more adsorbed noble gases than did the Earth, which in turn was formed of material with a higher noble gas content than Mars. In contrast, they suggested that the incorporation of volatiles such as CO_2, N_2 and H_2O was essentially independent of position. Assuming equal outgassing efficiencies for ^{14}N and ^{36}Ar on Mars, they deduced that the original $^{14}N/^{36}Ar$ on Mars was 40 times that on the Earth. Then, from the amount of Ar and N outgassed, they deduced that Mars had outgassed less efficiently than the Earth, and that 80-160 m of water were present at the surface. Thus estimates from the composition of the atmosphere suggest that Mars has outgassed from 6 to 160 m of water, proportionately 20-500 times less than the Earth.

Geological evidence

The surface of Mars has a variety of features that suggest it is water or ice rich. These include pitted ground that resembles terrestrial thermokarst, polygonally fractured ground, debris flows, craters with central pits and craters with lobate ejecta patterns[4,5,38,39] (Fig. 1). But channels provide the most obvious evidence for large amounts of water close to the surface. Immediately upon their discovery in 1972, the channels were recognized as probably having been formed by running water[20,21,40]. This interpretation is now almost universally accepted, although ice action and mass wasting may have been contributing factors in many cases. Alternative proposals that do not involve ice or water, such as faulting, and erosion by wind, lava or liquid hydrocarbons are all implausible for a wide variety of reasons[41,41]. Three main types of channels have been recognized: outflow channels, valley networks and fretted channels[42]. Because they lack tributaries, start full size, and have numerous attributes in common with terrestrial flood features, most of the outflow channels are believed to have been formed by large floods[40,41,43,44]. Some of the floods must have been enormous, having scoured swaths of terrain tens to hundreds of kilometres wide (Fig. 2). From the channel dimensions, Baker[41] estimates that discharges ranged as high as $3\times10^8\ m^3\ s^{-1}$. For comparison, the average discharge of the Mississippi is $3\times10^4\ m^3\ s^{-1}$. The cause of the floods is uncertain, and all probably did not form in the same way. Among the possibilities are volcanic interaction with ground ice[45,46], geothermal melting of ground ice[20], geothermal decomposition of hydrated minerals[47], and eruption of water under high pressures from confined aquifers[48]. All the plausible mechanisms imply abundant subsurface water either as ice or liquid. Because of the large discharge rates, these channels could form under present climatic conditions[49].

The total volume of water that flowed through the channels is difficult to determine, but rough estimates can be made from the amount of erosion that has been achieved. The largest outflow channels are round the Chryse basin, where about $5\times10^6\ km^3$ have been eroded to form channels, chaotic terrain and some of the smaller canyons[49]. The main east-north-east to west-south-west canyon was ignored in this estimate because it appears to be formed in large part by faulting[50]. The estimates of the eroded volumes could be in error by as much as a factor of two because of uncertainties in the channel depths. The minimum amount of water required to perform this erosion is estimated to be $7.5\times10^6\ km^3$ on the assumption that all the water carried its maximum sediment load, or 40% by volume[51]. This is a conservative assumption, implying that the terrain offered little resistance to erosion, and that most of the eroded sediment was transported as wash load at very high concentrations. The amount of water is equivalent to 50 m spread over the whole planet.

To determine what this volume implies about the total surface inventory, we must make some judgement about whether outflow channels started at specific locations because that was where water was especially abundant, or whether they started there because conditions necessary for rapid release were achieved. The distribution of valley networks suggests the latter is more likely. The valley networks resemble terrestrial river valleys in that they increase in size downstream and form integrated drainage networks (Fig. 3). They appear to have formed by slow erosion rather than by floods[41],[52,53], and largely as a result of groundwater sapping rather than surface runoff[54]. About two-thirds of Mars is covered by an ancient cratered upland that is almost certainly underlain by an impact-generated megaregolith. By analogy with the Moon, this probably has a high porosity, and consequently a large potential capacity for ground water[55]. Most of the valleys are incised into the ancient cratered upland and appear themselves to be very ancient[52,53,56,57], although there are rare exceptions[58]. Because of the difficulty of maintaining small streams under present climatic conditions, the valley networks have been taken as evidence that climatic conditions were significantly different early in the planet's history[59,60]. The distribution of the valley networks is almost uniform throughout the cratered terrain[52,53]. If formed mostly by groundwater sapping, they indicate that groundwater (or ground ice) was present everywhere in the cratered uplands very early in the planet's history.

I have suggested that floods were common in the region south and west of the Chryse basin because the large surface relief to the west of Chryse caused migration of ground water below the permafrost[48]. The consequent build-up of hydrostatic pressures caused catastrophic breakout from below the permafrost to form the floods. From the local relief and from the chaotic terrain, which provides evidence of withdrawl, we can outline the area that was drained to form the floods, and this constitutes about one tenth of the planet's surface[49]. Assuming, on the basis of the distribution of channel networks, that ground water was initially distributed evenly, we can extrapolate the volume of ground water released to form the Chryse channels to the entire planet, thereby obtaining a planet-wide inventory of 500 m. This figure is conservative because of the maximum sediment load assumption and because it is unlikely that all the ground water in the Chryse region came to the surface to form floods. The estimate would, however, be invalid if there were some re-cycling mechanism whereby the same water participated in the flooding several times[14].

Almost all of the outflow channels terminate in low-lying plains at high northern latitudes. These plains have numerous features that have been attributed to the presence of ground ice[4,38,39]. Included are polygonally fractured ground, irregular depressions that resemble terrestrial alases, table mountains similar to those in Iceland, and arrays of sub-parallel ridges and pits. The flood-waters probably pooled and froze in these areas. Most of the water may still be present as ice, protected by a debris cover that accumulated as the sediment-laden ice near the surface sublimed.

The above argument assumes that the water that eroded the Chryse outflow channels erupted from below the permafrost, estimated to be nominally 1 km thick. The amount of water existing as ice at shallower depths can be estimated from features in the fretted terrain and from 'terrain softening'. Fretted terrain is the term applied to areas where relatively flat lowlands are separated from the cratered uplands by a complex pattern of escarpments[61-63]. The main occurrence is where the boundary between the cratered uplands and the younger plains crosses the 30-50° N latitude band. Debris flows extend up to 30 km from the base of escarpments throughout the terrain (Fig. 4). In this latitude band, talus apparently moves away from slopes as a debris flow, instead of accumulating as a stable deposit at the base of slopes, as it does elsewhere. The simplest explanation of the enhanced mobility is that at the higher latitudes the talus contains ice[62,63], and that the ice is derived from the cratered uplands that are being eroded. Presence of abundant ground ice at high latitudes is also indicated by a general muting of the topography, which is also observed mainly in the 30-50° latitude bands[64] (Fig. 5). Here, crater rim crests are rounded, fine texture on ejecta blankets is lost, and the terrain between craters has a smooth rolling character rather than the crisply defined ridges and hills found at lower latitudes. Again, the most probable explanation of the softening is that it is caused by creep of the near-surface materials as a result of the presence of ground ice. Absence of debris flows and softening at low latitudes result from instability of ground ice at these latitudes and its consequent loss by sublimation. Absence of debris flows and softening at very high latitudes may result from the higher effective viscosity of ice at the low ground temperatures there[63,64]. The amount of ice required to mobilize the talus and to cause the softening is unclear, but analogy with terrestrial rock glaciers, and arguments based on the requirement that flow of ice rather than friction between the rock component controls the viscosity, suggests at least 10% ice is required[49,63]. If no ice is present at latitudes less than 30° and 10% is present at higher latitudes, then the upper 1 km contains 50 m of unbound water averaged over the whole planet. The geological evidence suggests, therefore, that at least 550 m of water have outgassed, substantially more than the estimates from the composition of the atmosphere, particularly those based on the noble gases.

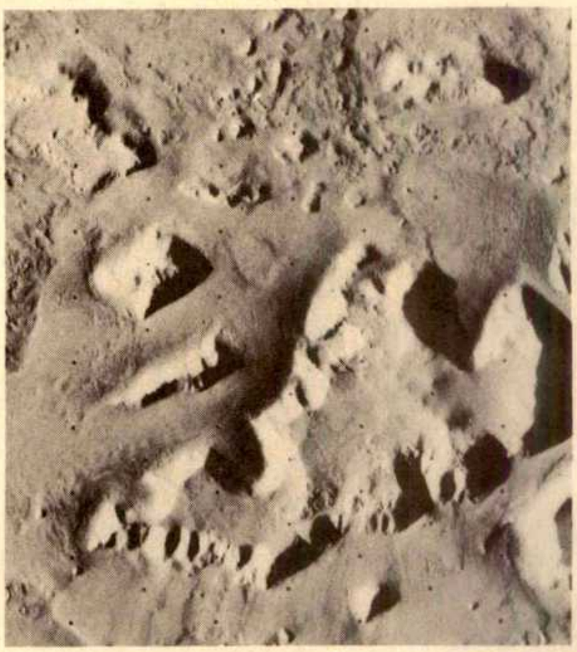

Fig. 4 Debris flows surrounding remnants of the cratered uplands at 45° N, 322° W. The mobility of the flows is thought due to entrainment of ice shed from the cliffs at their source[64]. The picture is 90 km across.

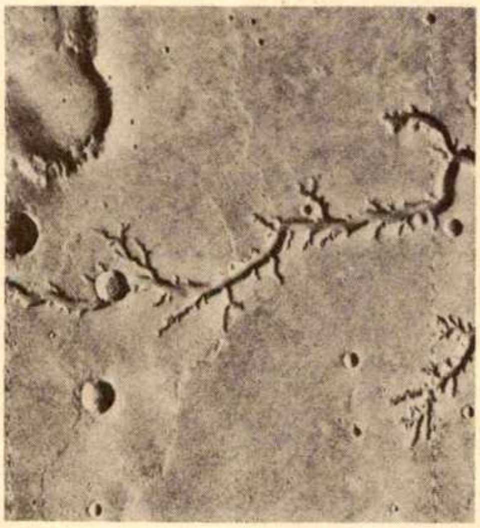

Fig. 3 The upper reaches of Nirgal Vallis, believed to have been formed by slow erosion of running water. The open nature of the tributary system, the lack of dissection between major branches, and their alcove-like terminations suggest origin by groundwater sapping. The scene is 80 km across.

SNC meteorites

The SNC meteorites provide yet another way of estimating the amount of water at the Martian surface. The basaltic composition of these meteorites, their young ages, and the composition of included gases indicate that they almost certainly come from Mars[65-67]. Assuming that they did, Dreibus and Wänke[68] show that most of the moderately volatile elements (Na, K, Rb, Zn, Ga, Cl, Br, I) are enriched in the Martian mantle with respect to the Earth's by factors that range from 1.3 to 4. Unfortunately, the SNC meteorites give little direct information on highly volatile compounds such as CO_2 and H_2O. To estimate these volatiles, Dreibus and Wänke invoked an accretionary model previously used to estimate the bulk composition of the Earth[69]. Following Ringwood[70], they suggest that the Earth and Mars accreted from two components, a highly reduced, volatile-poor component *A*, similar to enstatite chondrites, and an oxidized, volatile-rich component *B*, similar to Cl carbonaceous chondrites. The composition of the Earth's mantle is best matched by an *A*/*B* mixture of 85/15, whereas the moderately volatile elements in the Mars mantle imply it was formed from 65/35

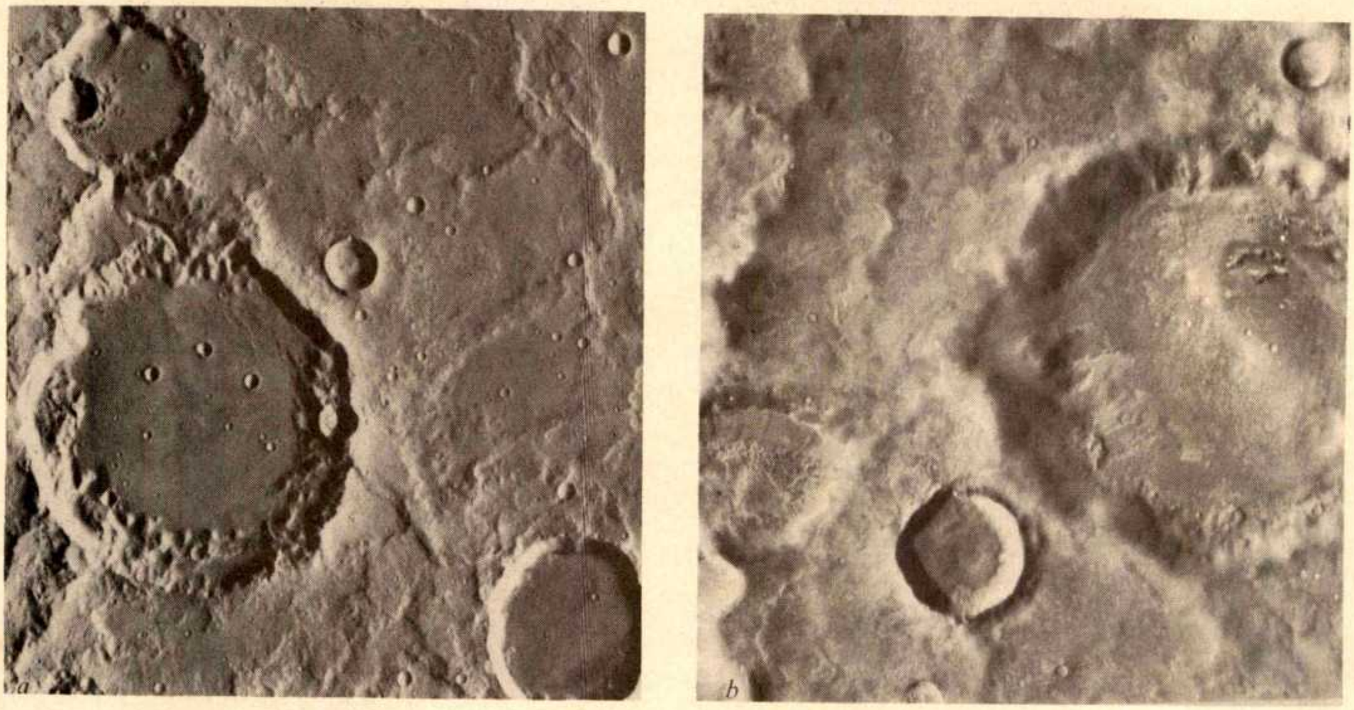

Fig. 5 Latitudinal contrasts in terrain morphology. *a*, Typical cratered upland at low latitudes (12° S). Rim crests of large craters are sharply defined, the small crater population is well preserved, and ridges between craters are clearly discernible. *b*, Typical cratered upland in the 30–55° latitude bands (48° S), showing terrain softening. Rim crests of large craters are rounded, the small crater population is poorly preserved and ridges between craters are muted. Both pictures are about 60 km across. The contrast between the two scenes has been ascribed to flow of the surface materials because of the presence of ground ice at the higher latitudes[63].

mixture. The bulk composition implied by such a mixture matches well the composition inferred from the mean density and moment of inertia[71]. Dreibus and Wänke conclude therefore that Mars was initially a very volatile-rich planet, having incorporated over twice the fraction of the volatile-rich component as the Earth. They suggest, however, that Mars retained very little of its initial, very large water inventory, because the planet accreted homogeneously, and the water in component *B* reacted with iron in component *A* to produce hydrogen, which was lost. In contrast, the Earth retained a large fraction of its water because it accreted inhomogeneously as is indicated by incompatibilities between the core and the upper mantle[70]. Part of component *B* apparently was added too late to the Earth to react with component *A*. Assuming no water on the surface of Mars at the end of accretion, Dreibus and Wänke estimate, from the solubility of water in basaltic magmas, that the global water inventory is 51 m. The fraction that has outgassed and is now at the surface is unknown but their favoured value is 10–20 m.

Current views and conclusions

We have just seen that estimates of the amount of water outgassed from Mars range over two orders of magnitude. The amount has major climatic implications, for if the lower figures are correct, then the planet has probably outgassed only about 0.1 bar of CO_2 and can never have experienced warm, wet, climatic conditions. If the upper figures are correct, then over 10 bars of CO_2 are likely to have outgassed, the planet may well have experienced warm, wet, conditions, and several biological implications may follow[72]. The geological evidence suggests that the lower estimates, those less than 100 m are extremely unlikely. The cumulative evidence of water erosion and ice-abetted processes is overwhelming[4,5,39,41,42], even if computation of the amount from the geological evidence is suspect. How then can we reconcile the low estimates with the geological evidence? The lowest estimates are based on the SNC meteorites and noble gases in the atmosphere. The Dreibus and Wänke arguments for Mars being initially volatile rich are convincing, but the supposition that Mars has lost all of its enormous initial inventory of water rests on the very questionable assumption of a complete planet-wide equilibration of its accreted components at the end of accretion. We know this is not true for the Earth[70], and it seems unreasonable to insist that it be true for Mars. If one per cent of component *B* were accreted sufficiently late that it could not react with component *A*, then geological requirements for hundreds of metres of water could be satisfied.

Arguments for low water abundances based on the noble gases in the atmosphere rest on two questionable assumptions, that the materials that formed the terestrial planets had a uniform ratio of noble to non-noble gases and that all the noble gases outgassed from the planet are still present in the atmosphere. We have seen from the Venus data that the first assumption is probably invalid. The second assumption is also suspect. Mars could have lost significant amounts of its early atmosphere by blow-off during large impacts[73]. The efficacy of such a process would depend on planet size, being more efficient on a planet like Mars, which has a relatively small gravitational field, than on the Earth. Loss would also preferentially favour the less reactive volatiles such as nitrogen and the noble gases, which tend to remain in the atmosphere, rather than volatiles such as CO_2 and H_2O, which tend to be combined with surface materials. Part of the early atmosphere could also be lost by hydrodynamic escape[73,74] during the early massive hydrogen loss referred to by Dreibus and Wänke[68]. Thus the very low estimates of the water inventory appear doubtful on grounds other than their clear incompatibility with the geological evidence. The somewhat larger estimates of the water inventory from the nitrogen isotopes and from the accretion model of Pollack and Black are also suspect if impact erosion of the atmosphere has been significant. Blow-off would favour nitrogen over water, so that a common step in both arguments, use of the terrestrial N_2/H_2O ratio to compute the water inventory from the reconstructed nitrogen inventory, will lead to an underestimate of the amount of water present.

In summary, data from the SNC meteorites suggest that Mars had an initial global inventory of volatiles that was proportionately larger than the Earth's. A major uncertainty is the fraction of these volatiles that has been outgassed and retained at the surface. Geological evidence suggests that at least 0.5 km of water have outgassed. On the reasonable assumption that CO_2, H_2O and N_2 were outgassed in terrestrial proportions, then 10–20 bars of CO_2 and 0.1 to 0.3 bars of N_2 also outgassed. Such a model can be reconciled with the geochemical data if the noble gases, and to a lesser extent nitrogen, were preferentially lost from the early atmosphere with respect to water. A plausible mechanism is impact erosion of the atmosphere. A large fraction of the water is present now as ground water and ground ice, well below the surface. Unbound water is present close to the surface only at high latitudes. Since the geological record emerged around 3.8×10^9 yr ago, the water has undergone some redistribution. That lost from the near surface materials at low latitudes by diffusion and seepage is probably now largely in the polar layered terrains. That involved in the formation of the large flood feature may still be at shallow depths in low-lying, high-latitude, northern plains. Indeterminate amounts of water may also be combined in weathering products, particularly clays. Because of the large amounts of CO_2 that were outgassed, early Mars may have possessed a thick atmosphere and experienced relatively warm climatic conditions. However, the fixation of CO_2 as carbonates caused the atmosphere to thin and surface temperatures to fall very early in the planet's history. Present climatic conditions are probably typical of what the planet has experienced for most of its history.

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ARTICLES

Multi-channel seismic imaging of a crustal magma chamber along the East Pacific Rise

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A reflection observed on multi-channel seismic profiles along and across the East Pacific Rise between 8°50′ N and 13°30′ N is interpreted to arise from the top of a crustal magma chamber located 1.2–2.4 km below the sea floor. The magma chamber is quite narrow (<4–6 km wide), but can be traced as a nearly continuous feature for tens of kilometres along the rise axis.

THE presence of a shallow crustal magma chamber at active spreading centres is an important element of current geological models for the formation of ocean crust[1–3]. However, the shape, longevity and along-strike variability of ridge-crest magma chambers remain the subject of considerably controversy[4,5]. The feasibility of using modern multi-channel seismic techniques to image magma chambers at ridge crests was demonstrated a decade ago when three 24-channel seismic reflection profiles were obtained across the East Pacific Rise (EPR) near 9° N[6,7]. In May–June, 1985, we returned to this area and carried out a much more extensive two-ship multi-channel seismic survey of the EPR between 8°50′ N and 13°30′ N. This experiment was designed to provide new information on the shape and dimensions of the axial magma chamber, and to investigate how the magma chamber varies along the rise axis over distances of tens to hundreds of kilometres.

The geological and tectonic setting of the EPR in this area has been well-established from Sea Beam and Sea MARC I surveys[8-10] extensive dredging along the rise axis[11,12], and very high-resolution investigations using bottom photography and manned submersibles[13]. This portion of the EPR is spreading at relatively fast rates of 5-6 cm yr^{-1} (half-rate)[14] and contains one major transform fault (the Clipperton at 10°15′ N), two large overlapping spreading centres (OSCs) at 9°03′ N and 11°45′ N and several smaller non-transform offsets including OSCs and other small deviations in axial linearity (Devals)[9-11]. Our seismic survey was primarily concentrated in two areas—between the 9°03′ N OSC and the Clipperton transform, and in a second area just north of the 12°54′ N OSC (Fig. 1). Seismic refraction data are available in both areas indicating the existence of a crustal low velocity zone (LVZ), interpreted as a magma chamber[15-18], at the rise axis. However, our survey also encompasses part of the ROSE refraction area (~11°30′ N to 12°30′ N) where evidence for a large crustal magma chamber is generally lacking[19,20].

The experiment involved two ships, the *Robert D. Conrad* of Lamont-Doherty Geological Observatory and the *Thomas Washington* of Scripps Institution of Oceanography. More than 3,500 km of conventional, 48-channel common-depth-point (CDP) reflection data were obtained including 30 new profiles across the EPR and a series of CDP reflection lines along the crest of the EPR between 8°50′ N and 13°30′ N (Fig. 1). The seismic velocity structure of the crust and upper mantle in the axial region was determined using the two-ship expanding spread profiling (ESP) technique[21]. The ESPs were oriented parallel to the rise axis and shot using airguns and, in some cases, explosives. In the 9° N area, ESPs were shot on the rise crest, and at two and five km away from the ridge crest on either side. In the 13° N area, five ESPs were located within ±3.6 km of the rise axis.

Single-ship stacked time sections form the basis of our preliminary observations. About two-thirds of the cross-axis lines have been migrated after stack using the wave equation technique in the F-K domain[22]. Analysis of the ESPs has mainly involved P-wave velocity travel-time inversions on the airgun portions of the ESPs. An initial velocity-depth function was constructed using the tau-sum technique after transformation of the data into tau-p[23]. The velocity-depth function was then iteratively refined by forward travel-time and WKBJ synthetic seismogram modelling.

Results

Two representative CDP reflection profiles across and along the EPR are shown in Figs 2 and 3. A large amplitude reflection is present about 0.6 s below the sea floor on both profiles. On the cross-ridge profile, this high-amplitude event is centred beneath the rise axis and is less than 3 km in width, although a weak continuation of this reflector appears to extend beneath the ridge flanks, especially west of the rise axis. An event is present at about 6 s two-way travel time that may be a Moho reflection based on its position in the section. On the reflection profile shot along the rise axis, a large-amplitude, relatively flat-lying reflector is present at about 4 s two-way travel time that corresponds to the event seen on the cross-ridge profiles. This event can be traced for distances of tens of kilometres along the crest of the EPR, and in a few locations, the amplitude of this reflector is strong enough that it can be observed on single channel monitor records. Some weaker, low frequency events are present below this strong reflection that may also be real, but no obvious Moho reflection is observed.

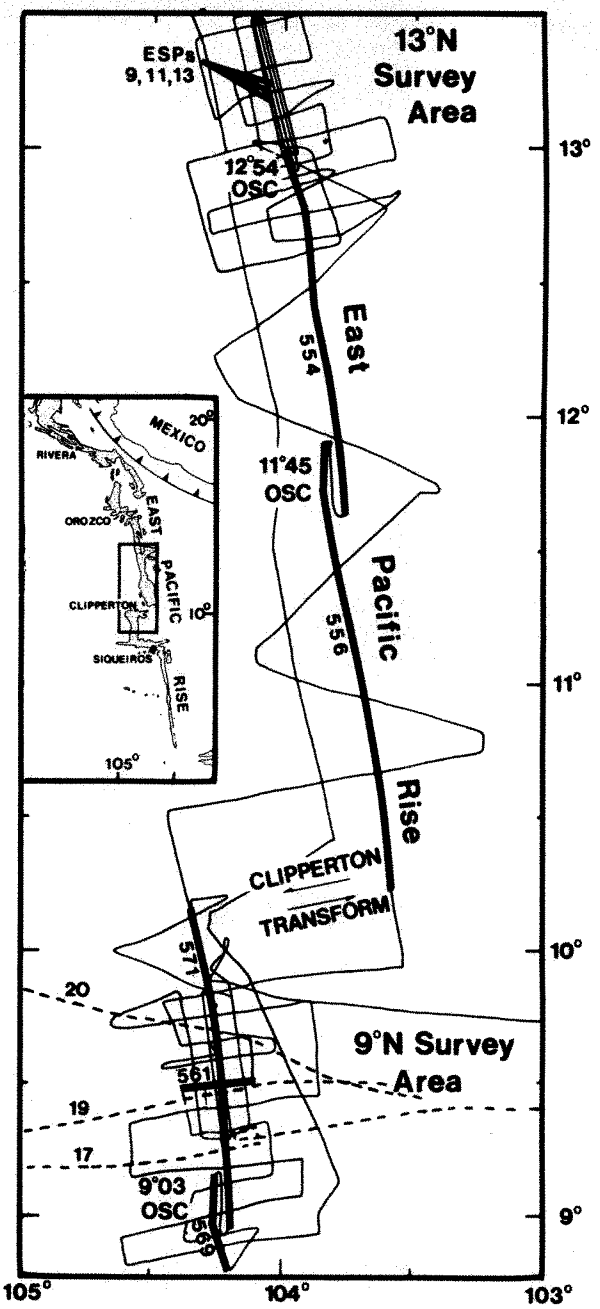


Fig. 1 Map showing the East Pacific Rise (EPR) and the track coverage obtained during our 1985 multi-channel seismic experiment. Labelled profiles (thick solid lines) are shown in Figs 2 and 3 or interpreted in Fig. 5. Dashed lines show the location of the reflection profiles described by Herron *et al.*[6,7].

Evidence for a crustal magma chamber. Several lines of evidence indicate that the shallow event seen in these records at the rise axis is a reflection from the top of a crustal LVZ. The reflection is seen on single channel monitor records and in common mid-point gathers and thus is not an artefact of the stacking process. The stacking velocity of this event (2.3-2.4 km s^{-1}) is much higher than the value of 1.5 km s^{-1} expected for a water-path sidescatter event or a high-order water bottom multiple. This stacking velocity yields an interval velocity of 4.9 km s^{-1} for the crust above this reflector which is a reasonable average value for the upper 1 km of the oceanic crust near the EPR[25]. At several locations along the ridge, the polarity of this event observed on the near-offset trace in CDP gathers is clearly reversed relative to the sea floor return, as would be expected for a reflection from the top of a LVZ. However, the waveform

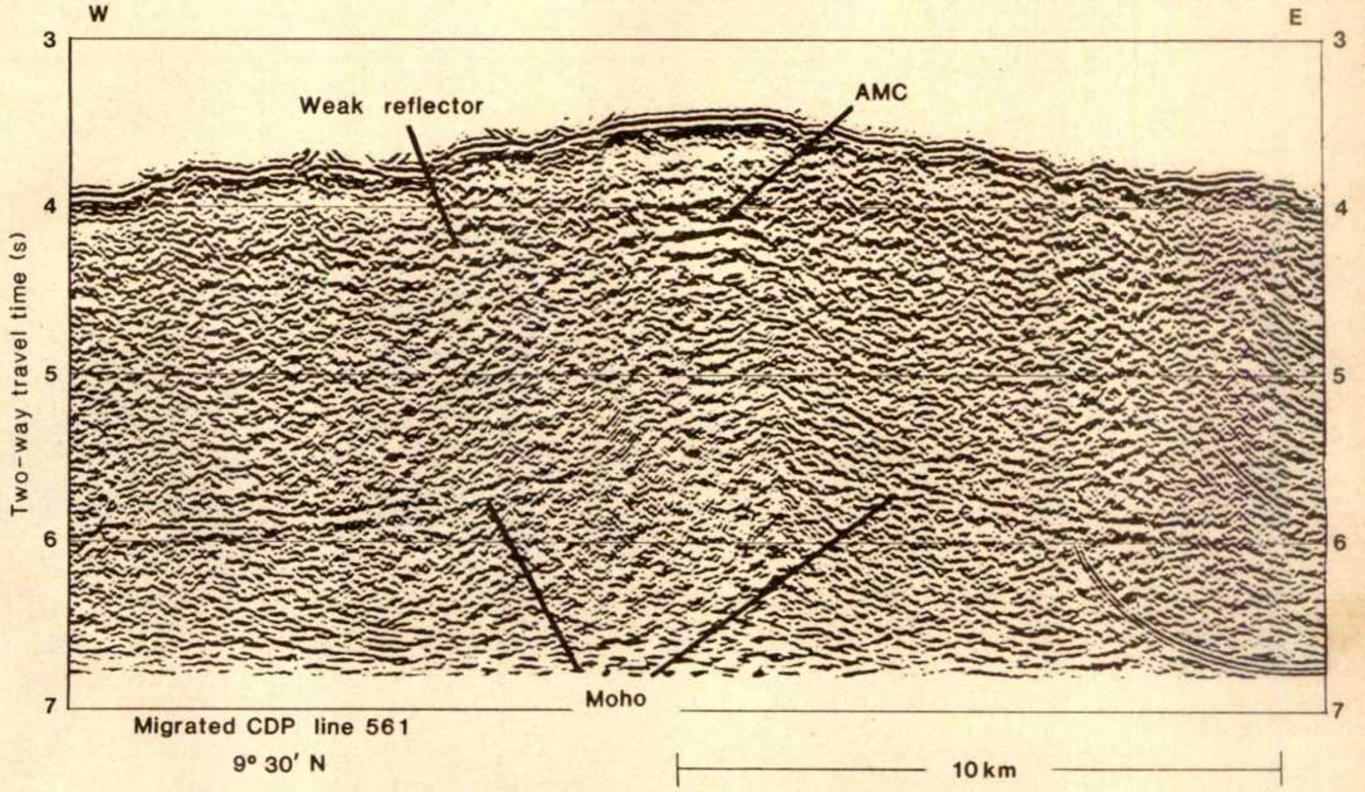


Fig. 2 Migrated time section for CDP Line 561 which crosses the EPR near 9°30′ N. These data were shot using a 29.3 l (1785 in^2) airgun array and recorded on a 48-channel, 2.4-km streamer with an active group length of 50 m. The data were bandpass filtered 5–40 Hz, corrected for spherical divergence, predictively deconvolved (filter length 250 ms; prediction distance 40 ms), stacked and migrated using the F–K wave equation technique[22]. The data are displayed with a time variable gain and a 3-trace additive mix. The relatively flat, high-amplitude event beneath the rise axis is believed to be a reflection from the top of an axial magma chamber (AMC). A weak event at the same depth as the AMC reflector can be traced out beneath the ridge flanks and could represent the top of a frozen chamber. Moho reflections can be traced within 2–3 km of the rise axis.

is quite variable, even from CDP to CDP, and in other locations a phase-reversal is not apparent. The strongest evidence that this event is associated with an axial LVZ comes from the ESPs shot along the rise axis. They display a pronounced shadow zone for crustal arrivals beyond 11 km range that is very diagnostic of a LVZ[18] (Fig. 4). Although ESPs were obtained at only two locations along the ridge (9°35′ N and 13°10′ N), sonobuoys collected elsewhere along the rise axis also frequently display this same strong attenuation of refracted energy beyond 10–12 km range.

We interpret this LVZ to be an axial magma chamber (AMC) or zone of melt within the crust beneath the rise axis and use the reflections from the top of this LVZ to infer the width, depth and along-axis variation in the magma chamber.

Width of the magma chamber. The results from this experiment provide very tight constraints on the width of the magma chamber at the EPR. The section shown in Fig. 2 appears to be typical of the rise crest south of the Clipperton transform. The reflection from the top of the magma chamber migrates into a relatively flat, high-amplitude event with a maximum width of 2–3 km. We have not yet been able to image either the walls or floor of the magma chamber, but Moho reflections can be confidently traced to within at least 2–3 km of the rise axis on both the eastern and western flanks of the ridge, placing an upper bound of 4–6 km on the total width of any crustal magma chamber along this portion of the EPR. A weak, discontinuous reflector can be traced out onto the ridge flanks at the same depth as the magma chamber reflection that could represent the top of a frozen magma chamber, perhaps marking the contact between the sheeted dykes and the underlying isotropic gabbros. This is the first time, as far as we are aware, that an event such as this has been seen in marine reflection profiles.

In the 13° N area, a magma chamber reflection is observed on only a few profiles crossing the ridge, and Moho reflections are generally not seen, possibly due to somewhat rougher ridge-flank topography. Where magma chamber reflections are observed, they appear to be no more than 2 km wide. The existence of a very narrow crustal magma chamber near 13° N is strongly supported by an analysis of the three ESPs shown in Fig. 4. Although a seismic LVZ is present 1.2 km below the sea floor at the rise crest (ESP 9), only 3.6 km away (ESP 13) a typical oceanic velocity structure is present with a well-developed lower crustal (layer 3) refractor and no evidence for a crustal LVZ. Even at ESP 11, 1.5 km from the rise crest, arrivals with phase velocities of 7 km s^{-1}, characteristic of the lower crust, are clearly seen beyond 9–10 km range. However, these arrivals are not continuously connected with arrivals at shorter ranges having phase velocities typical of the upper crust (layer 2). The existence of this small shadow zone indicates the presence of a thin (<300 m thick) LVZ sandwiched between the upper and lower crust. However, the total width of the magma chamber in this area is probably less than 4–6 km, and only 1.5 km from the ridge axis the seismic LVZ, if present at all, is extremely thin.

Our estimate of the width of the magma chamber at the EPR is thus well-constrained by the width of the reflections from the roof of the chamber, the gap in the Moho reflection at the rise axis and the crustal velocities from ESPs in the axial region. The narrow magma chamber we have found (4–6 km maximum, generally <3–4 km wide) is incompatible with the large (>10–20 km), funnel-shaped bodies proposed on the basis of some ophiolite models[3,26], but is consistent with the results of recent refraction experiments at the EPR[17–19].

Depth of the magma chamber. The depth of the top of the magma chamber is also well-constrained in our experiment by the ESPs shot along the rise axis and the two-way travel time between

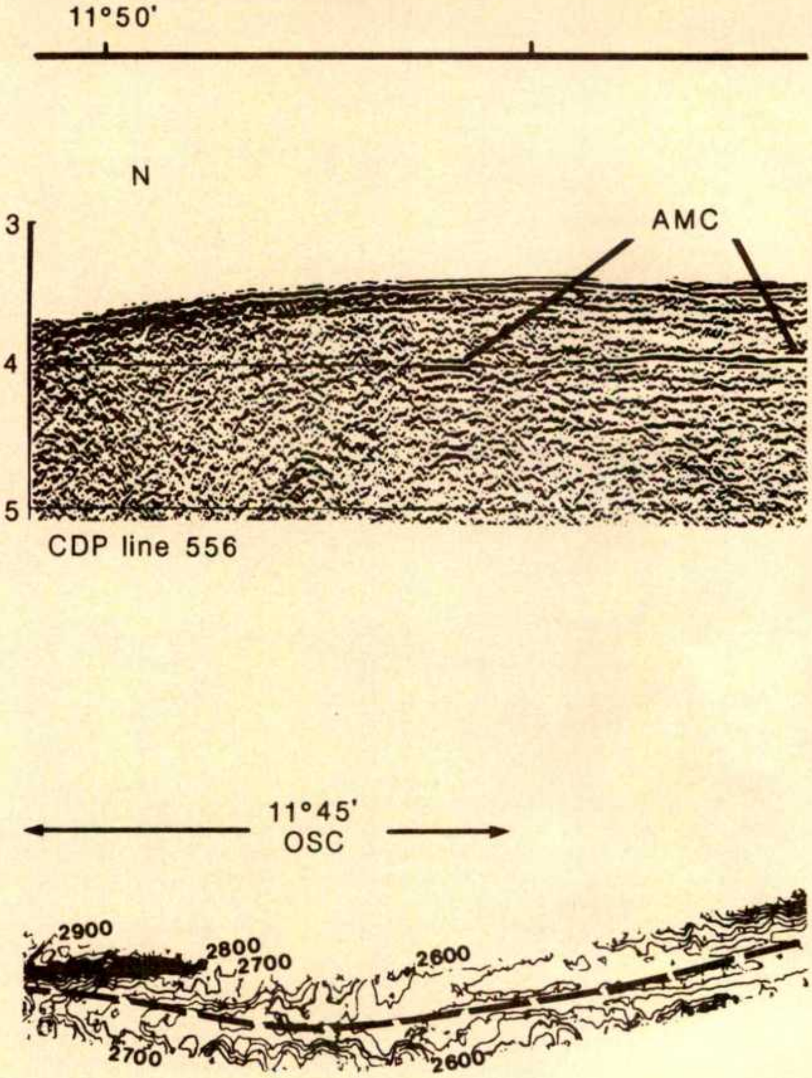
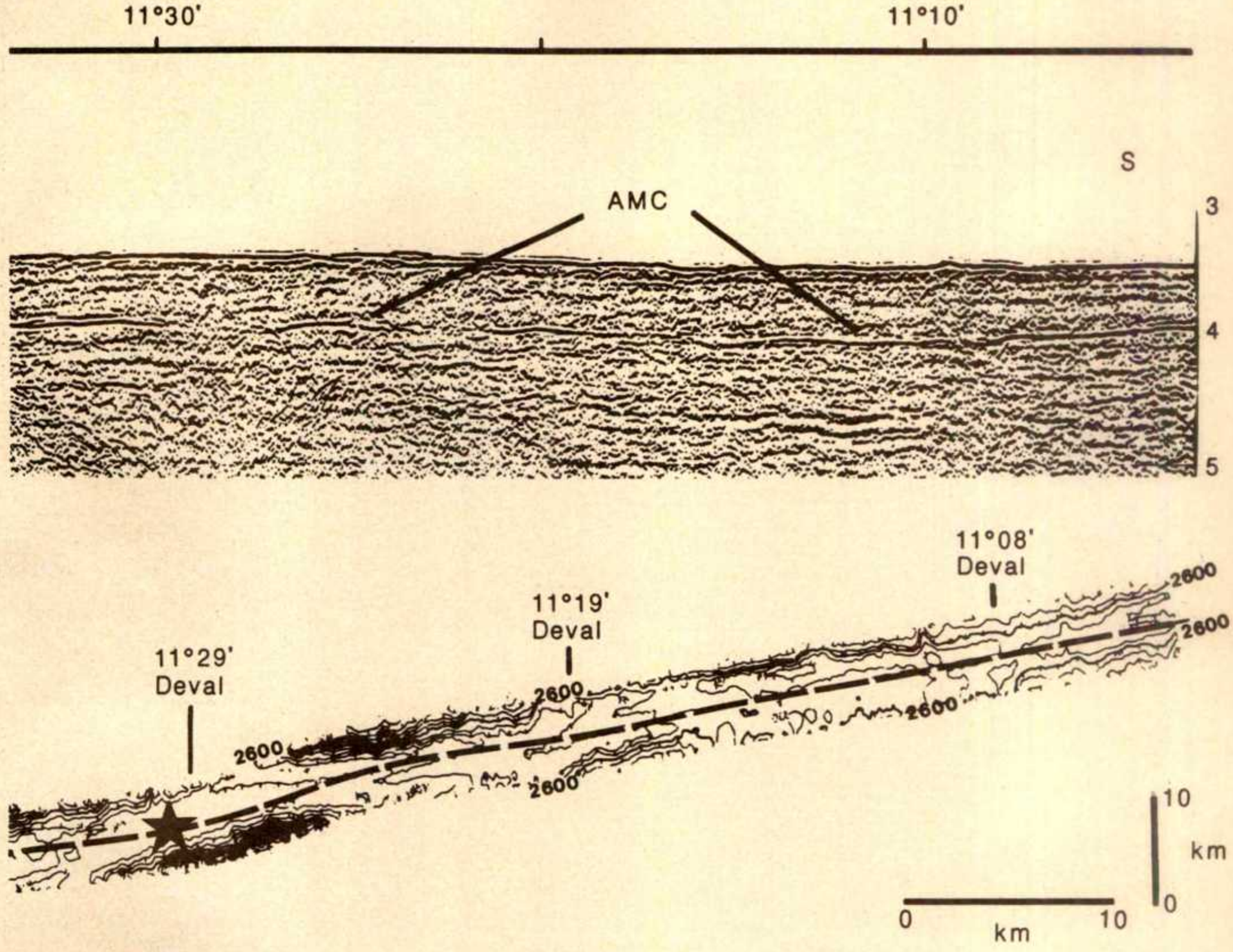


Fig. 3 Time section of a 100-km-long segment of CDP line 556 along the EPR south of the 11°45′ N OSC illustrating the along-strike continuity of the magma chamber reflection. Lower panel shows a 20-m Sea Beam map of this portion of the EPR crest and the location of the MCS profile (dashed line). The reflection data have been bandpass filtered 5–40 Hz, corrected for spherical divergence, predictively deconvolved (filter length 750 ms; prediction distance 40 ms) and filtered again 6–20 Hz. The data are displayed as in Fig. 2. Deval locations after Langmuir *et al.*[11]; stars indicate areas of reported hydrothermal activity[24].

the sea floor and the AMC reflection. Along the portion of the EPR we have surveyed, the roof of the magma chamber varies in depth between 1.2 and 2.4 km below the sea floor, a result which is consistent with previous estimates based on seismic refraction studies at the EPR[15–18]. The most interesting new observation made in this study is the correlation between the depth of the magma chamber (that is, the thickness of the crustal lid above the chamber) and the depth of the rise axis. Although the depth of the EPR varies <300 m along the 500 km of ridge we surveyed, the depth of the magma chamber changes by more than a kilometre. In general, the shallowest parts of the rise axis (south of Clipperton, between 11° N and 11°40′ N, north and south of the 12°54′ N OSC) are associated with the thinnest crustal lids, while the magma chamber reflection is deeper, more discontinuous and, in some cases, absent altogether where the rise axis is deep (north of Clipperton, near the OSC at 9°03′ N and 11°45′ N). These results are a striking confirmation of the inferences of MacDonald *et al.*[9] of magmatic budget based on axial geomorphology.

Along-strike variation in the magma chamber. As part of this experiment, we obtained a unique series of reflection profiles along the crest of the EPR between 8°50′ N and 13°30′ N, using Sea Beam to keep the vessel positioned atop the axial high. An AMC reflection is observed along slightly more than half (61%) of the 500 km of ridge crest we surveyed (Fig. 5). The most continuous magma chamber reflection is found south of the Clipperton transform, where a high amplitude event can be traced more than 90 km from just north of the large 9°03′ N OSC to a point about 10 km south of the Clipperton transform (line 571). There are three locations, dashed on the interpretive section, where the AMC event becomes weak and somewhat discontinuous. However, in each instance we believe this was caused by the vessel sliding off the axial high rather than a physical discontinuity in the magma chamber. Several other small gaps in the AMC reflection (for example, 11° N, 11°29′ N, 11°40′ N, 11°50′ N) may have a similar origin. Magma chamber reflections that are laterally continuous for 40–50 km are also observed south of the 12°54′ N OSC on line 554 and between 11° N and 11°40′ N on line 556. These results are the first geophysical evidence for the continuity of an axial magma chamber over distances of several tens of kilometres at the EPR.

The largest gap in the AMC reflection (~77 km) occurs north of the Clipperton transform. This ridge segment is deep (>2,700 m) and relatively narrow (<4 km wide), plunging over 200 m toward the Clipperton ridge–transform intersection (Fig. 5). The absence of an AMC reflection along this part of the EPR indicates that a magma chamber, if present at all, is much smaller than to the south. Seismic refraction studies had previously suggested the possible absence of a crustal magma chamber in the vicinity of ROSE line 6 near 11°19′ N[20]. We observe a clear, phase-reversed AMC event on line 556 in this area (Fig. 3). However, no corresponding event is seen on the cross-axis profiles, again suggesting that the magma chamber is quite small or partially solidified. The presence of a small, seismically unresolvable AMC along a substantial length of the ridge segment north of the Clipperton transform is the strongest



geophysical evidence so far that crustal magma chambers are not steady-state features at these spreading rates.

The AMC reflection deepens toward the large OSCs at 9°03′ N and 11°45′ N. It can be traced into the region where the spreading centres overlap, but in each case the magma chamber reflections disappear before reaching the tip of the overlapping ridges (Fig. 5). Reflection profiles across and through the overlap basins at both OSCs do not reveal an AMC reflection, suggesting that the axial magma chamber is physically disrupted. These observations, and the small dimensions of the magma chamber documented above, clearly favour the OSC model of MacDonald *et al.*[9,28]. However, both their model and Lonsdale's[29,30] model predict a continuous magma chamber beneath small-offset OSCs. We observe a gap in the AMC reflection across the small OSCs at 12°54′ N and 12°37′ N (Fig. 5), suggesting that the magma chamber is disrupted as predicted by Langmuir *et al.*[11] on petrological grounds. However, the AMC reflector is generally continuous across many of the smaller ridge axis discontinuities located between OSCs, including seven of the ten Devals identified along this portion of the EPR (Fig. 5). It may be significant that several Devals (9°17′ N, 9°53′ N, 11°08′ N, 12°28′ N) are located near or close to prominent dips in the AMC reflection, although in these areas it is difficult to keep a ship located precisely on top of the axial high.

Discussion

We have not yet been able to image either the walls or floor of the magma chamber, but some aspects of its shape can be constrained by our data. Figure 6 shows a model that is consistent with our observations. The magma chamber is modelled as a flat-topped body with a roof 2–3 km wide. We include a thin region of liquid melt in the top of the chamber to explain the high amplitude of the AMC reflector and its reversed polarity. However, both of these observations require only a strong, negative velocity contrast and do not necessitate the presence of a liquid. A chamber consisting, for example, of a partially solidified crystal mush[31] would also be consistent with our results. The observation of Moho reflections very close to the rise axis, and our ESP data, indicate that the chamber does not widen with depth but remains quite narrow.

One of the major remaining uncertainties concerns the thickness of the chamber. In Fig. 6 we envision a mushroom-shaped magma chamber with a bulbous shallow top and a narrower central stalk that extends to depths equivalent to Moho in the adjacent crust. We base this model on the assumption that, were the chamber much thinner (for example, a partially molten sill), unreasonable values of the elastic attenuation factor, *Q*, would be required to explain the complete extinction of arrivals beyond 11 km range on the ridge axis ESPs. However, we have no direct knowledge of the physical properties of the material within the chamber or its thickness and, consequently, the root structure of the magna chamber shown in Fig. 6 is still largely speculative.

There is a good correlation between some of the geochemical and petrological variations in basalts dredged from this portion of the EPR[11] and our seismic results. The basalts with the most homogeneous compositions and highest eruption temperatures

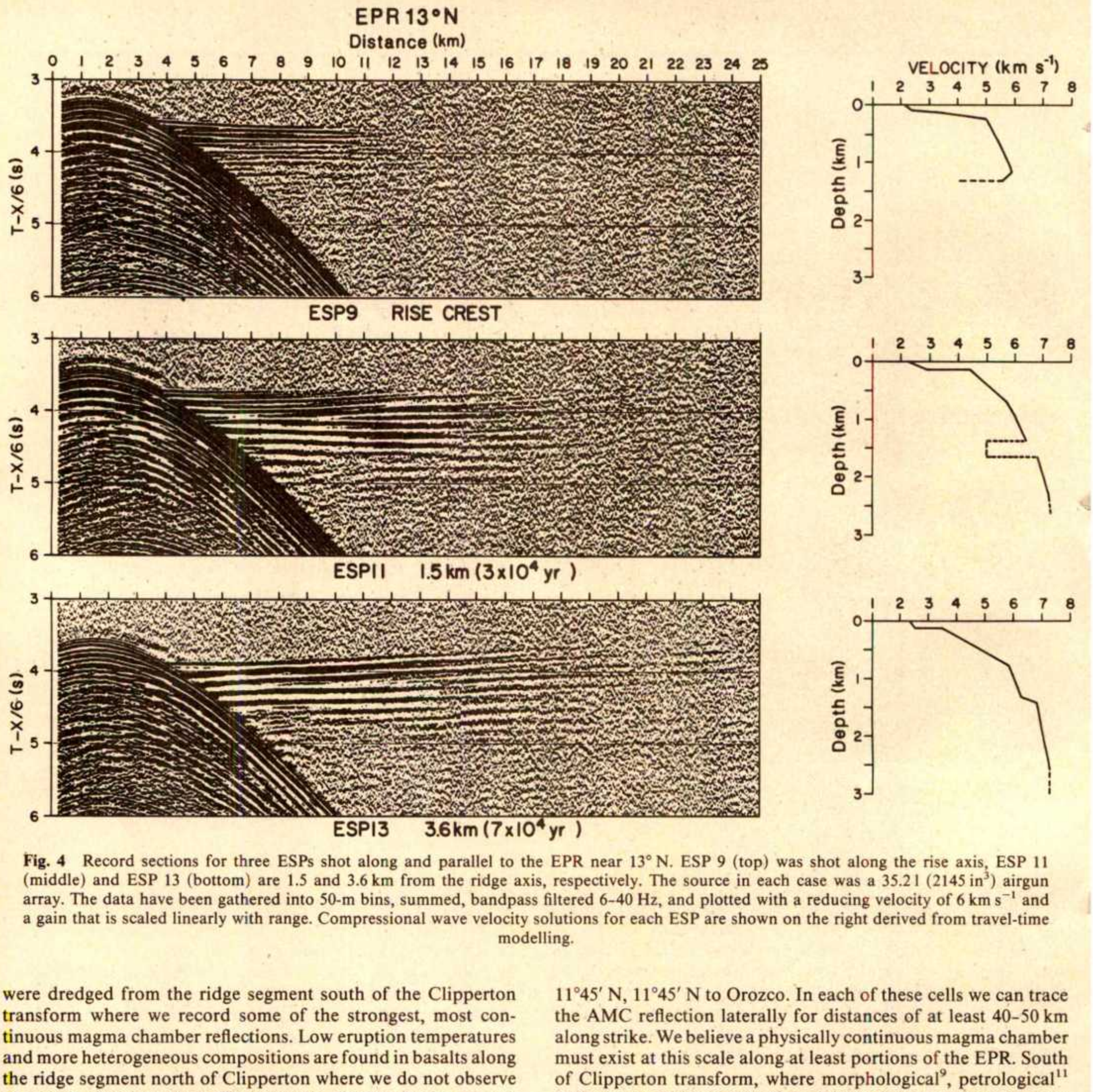


Fig. 4 Record sections for three ESPs shot along and parallel to the EPR near 13° N. ESP 9 (top) was shot along the rise axis, ESP 11 (middle) and ESP 13 (bottom) are 1.5 and 3.6 km from the ridge axis, respectively. The source in each case was a 35.2 l (2145 in^3) airgun array. The data have been gathered into 50-m bins, summed, bandpass filtered 6–40 Hz, and plotted with a reducing velocity of 6 km s^{-1} and a gain that is scaled linearly with range. Compressional wave velocity solutions for each ESP are shown on the right derived from travel-time modelling.

were dredged from the ridge segment south of the Clipperton transform where we record some of the strongest, most continuous magma chamber reflections. Low eruption temperatures and more heterogeneous compositions are found in basalts along the ridge segment north of Clipperton where we do not observe a magma chamber event. The gaps we observe in the magma chamber reflection across both large- and small-offset OSCs also appear to substantiate petrological evidence for a lack of magmatic continuity and mixing across OSCs[11]. However, the smaller offsets between OSCs, such as Devals, are generally not associated with breaks in the magma chamber reflection, and we can trace the AMC reflector continuously across Devals that appear to separate regions of distinctly different petrology (for example, 9°53′ N, 11°08′ N, 12°28′ N).

Based on the geochemical variability of basalts erupted along the ridge, Langmuir *et al.*[11] have recently argued for a much smaller scale magmatic segmentation of the EPR than previously proposed[32–34]. Instead of a single, centrally supplied magma chamber underlying each tectonically defined spreading centre cell, they propose that the AMC can be physically discontinuous within a single cell with multiple injection centres distributed along the ridge. We have seismic data on the along-strike continuity of the AMC reflection from three tectonically defined spreading centre cells: 9°03′ N to Clipperton, Clipperton to 11°45′ N, 11°45′ N to Orozco. In each of these cells we can trace the AMC reflection laterally for distances of at least 40–50 km along strike. We believe a physically continuous magma chamber must exist at this scale along at least portions of the EPR. South of Clipperton transform, where morphological[9], petrological[11] and seismic evidence all indicate a large, robust magma chamber is present, the entire spreading cell (>90 km) appears to be associated with a single, laterally continuous magma chamber. However, in the other two cells, significant gaps in the AMC reflection may indicate that the magma chamber is much more discontinuous along strike, although the absence of an AMC reflection does not necessarily preclude the presence of a magma chamber.

The picture that emerges from both the seismic and petrological data is of a magma chamber that is quite narrow (<4–6 km wide), but continuous for several tens of kilometres along the ridge axis. Where the magmatic budget is high (for example, south of Clipperton), a single magma chamber can extend along an entire, tectonically-defined spreading centre cell. The chamber will be relatively large and well-mixed, and erupting basalts will be geochemically homogeneous. Where the magmatic budget is low (for example, north of Clipperton), the magma chamber is smaller, or absent altogether, incompletely mixed and disrupted by both large and small OSCs. Lavas will be

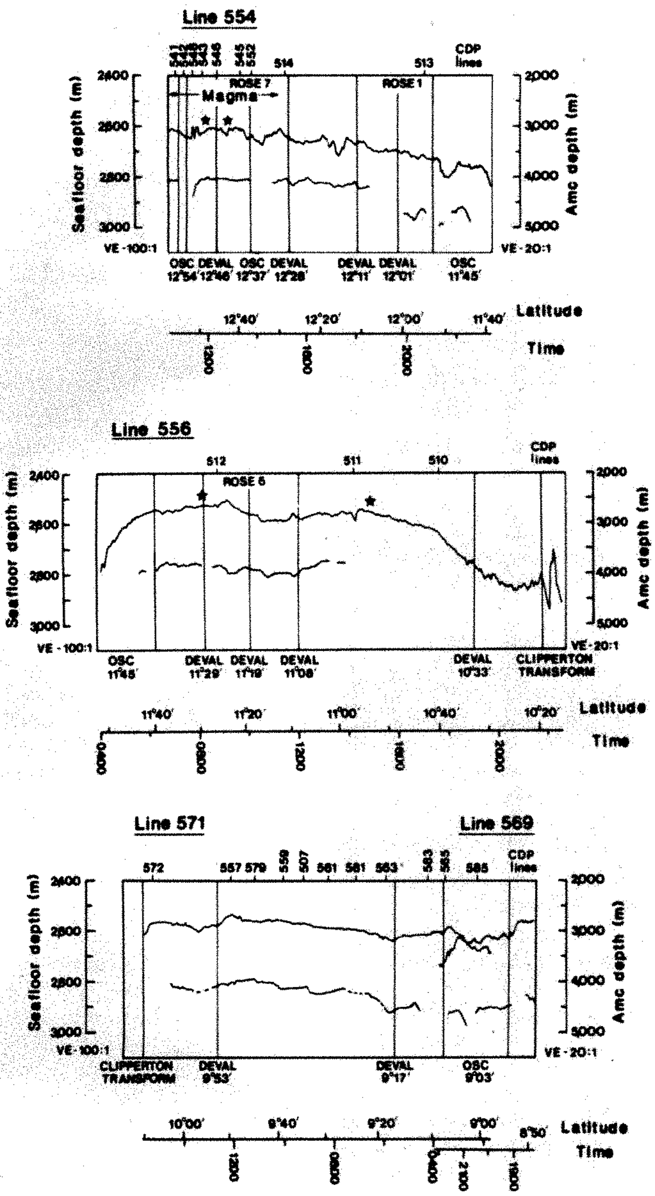


Fig. 5 (Left) Interpretive line drawing of a longitudinal section along the EPR extending from the 9°03′ N OSC to the Clipperton Transform (lower panel), from the Clipperton Transform to the 11°45′ N OSC (middle panel) and from the 11°45′ N OSC to the 12°54′ N OSC (upper panel). In each drawing the top curve, plotted using the scale on the left, is the sea floor depth derived from the centre beam of the Sea Beam swath. The lower curve, plotted using the scale on the right, is the depth of the AMC reflection below the sea surface derived from the observed two-way travel time assuming an interval velocity of 4.9 km s^{-1} for the crust between the sea floor and this reflector. Each panel is annotated with the location of the ridge crossing profiles (labelled ticks) and various ridge axis discontinuities including transforms, OSC and Devals (vertical bars). The stars mark the locations of known hydrothermal activity[13,24,27].

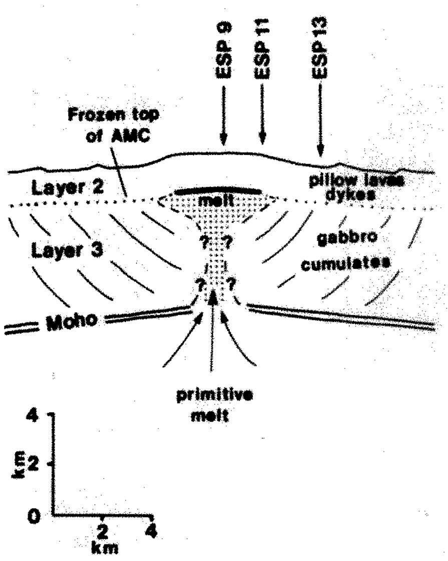


Fig. 6 Schematic drawing of the shape and dimensions of the AMC at the EPR based on the multi-channel seismic results presented in this paper. Location of ESPs shown in Fig. 4 is indicated for comparison. See text for discussion.

geochemically and petrologically diverse. Although smaller offsets between OSCs are not generally associated with physical disruptions in the AMC, geochemical anomalies associated with these features may reflect multiple regions of supply from below the crust or a magma chamber that is incompletely mixed and heterogeneous in its composition. The correlation of this morphological, geochemical and seismic variability along the rise axis appears to offer great promise for an improved understanding of the magmatic and tectonic processes occurring at the EPR.

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A family of unusually spliced biologically active transcripts encoded by a *Drosophila* clock gene

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Complementary DNA cloning of the transcripts of the Drosophila *clock gene* period *reveals three distinct transcripts. These result from unusual splicing pathways, one involving a CG 3′ splice site and one resulting in the use of two different reading frames in one exon, and they predict three separate proteins. Two of the cloned cDNAs can restore clock function to mutant arrhythmic flies.*

BIOLOGICAL clocks control periodic fluctuations (rhythms) of behaviour and physiological parameters in living organisms. The circadian clock of mammals (for review see ref. 1), which resides in the central nervous system and governs daily fluctuations of various physiological functions and patterns of behaviour, has been extensively studied from a physiological point of view. Molecular genetic aspects of clock function, however, have been studied in more detail in the fly *Drosophila melanogaster*.

The *period* (*per*) gene is one of the four loci in *D. melanogaster* that influence this fly's biological rhythms[2,3]. It is suspected to be directly involved in the oscillators underlying these rhythms for two reasons. (1) Whereas certain *per* mutations abolish rhythms (*per*^{o}), others modify their periodicities by shortening them (*per*^{s}) or lengthening them (*per*^{L})[2]; this indicates that the gene is not only essential for clock function but is involved in determining period length. (2) The *per* gene influences at least one clock function other than that which underlies circadian rhythms: the male's courtship song rhythm[4], which has a period length of only one minute and is affected by *per* mutations in a fashion that parallels the way in which they affect circadian rhythmicity.

Recently, genomic DNA from *per* has been cloned[5,6], introduced back into *per*^{o} mutant flies (by means of P-element mediated transformation) and shown to restore their circadian phenotype[7,8]. Analysis of RNA for *per* transcripts has identified a 4.5-kilobase (kb) messenger RNA which is expressed mainly in flies' heads[9], is absent from RNA of flies with deletions of the *per* locus and is restored after transformation with *per* DNA[8]. DNA sequence analysis and biochemical experiments indicated that *per* codes for a large protein that may have proteoglycan characteristics[10–12].

To characterize *per* and the putative protein(s) encoded by it further, we have now cloned complementary DNAs for transcripts originating at this locus. We show here that at least three transcripts are encoded by the *per* gene. These transcripts are generated by alternative splicing events, some of which are very unusual. Two of the cDNAs are also shown to be biologically active in restoring the circadian rhythm to mutant (*per*^{o}) flies. The structure and possible functions of the putative family of *per* proteins, as well as the unusual splicing events involved in generating them, are discussed.

Cloning of cDNA

Previous experiments using genomic *per* DNA as a hybridization probe for *Drosophila melanogaster* RNA indicated a major 4.5-kb transcript originating from the *per* locus[5,6]. We expected therefore to clone a 4.5-kb cDNA which hybridizes to the appropriate band on Northern blots. We constructed two cDNA libraries (see Fig. 1, legend) from which we cloned three types of cDNA corresponding to three species of messenger RNA, all of which appear to be ~4.5-kb long. The level of expression of the two minor mRNA variants (types B and C) is much lower than that of the major type A mRNA (see below). This fact, along with the size similarity of the three types, probably explains why mRNA heterogeneity was not detectable in earlier RNA hybridization experiments[5,6].

Type A—the most abundant

The most abundant type of cDNA is designated type A. Its relative abundance is estimated to be about 70%, as six of the eight clones (some of which are shown in Fig. 1*b*) that extend sufficiently to the 3′ end to allow for their classification are of type A. The structure of type A cDNA is shown in Fig. 1*b*. The entire *per* genomic DNA sequence and the predicted amino-acid sequence of the *per* A protein, as translated from type A cDNA, are presented in Fig. 2.

Jackson *et al.*[10] have recently published a sequence and organizational study of the *per* gene. Of the eight exons described in their study, six are essentially identical in sequence and position to those described here for type A cDNA (our exons 1, 3, 4, 6, 7 and 8 in Fig. 1*b*). But our exon 2 is absent from their description and our exon 5 is divided into two parts in the summary of their study. The absence of an intron in our exon 5 adds 35 amino acids to the predicted protein sequence, and the addition of exon 2 to the structure extends the N-terminus of our protein by 62 amino acids and places the initiating ATG at position 2,837, as indicated in Fig. 2. Because our analysis of the gene organization is based mainly on cDNA clones which cover the entire transcribed region, we believe that the structure which is presented (Figs 1*b* and 2; see legend to Fig. 2) is correct.

In summary, we predict for the major type A *per* transcript an RNA of 4,519 nucleotides in length (excluding the poly(A) tail), containing a 368-base pair (bp) untranslated 5′ region. The translated exons code for a 1,218 amino-acid protein and there are 497 untranslated nucleotides at the 3′ end. The predicted relative molecular mass (M_r) of the protein is 127,000 (127 K), in the absence of any post-translational modification.

Expression of type A mRNA was evaluated by Northern blot analysis using a probe that contains sequences shared by type A and C cDNAs (probe 4, Fig. 1*a*). (Note that all type A sequences are included in type C; see below and Fig. 1*b*.) As type A is much more abundant, probe 4 detects mainly the expression of type A mRNA. Figure 3*b* (lane H, upper band) shows that type A is mainly expressed in adult flies' heads; only very low levels of transcript are observed in body and embryo RNA (see lanes E, B, H). This result agrees with those from

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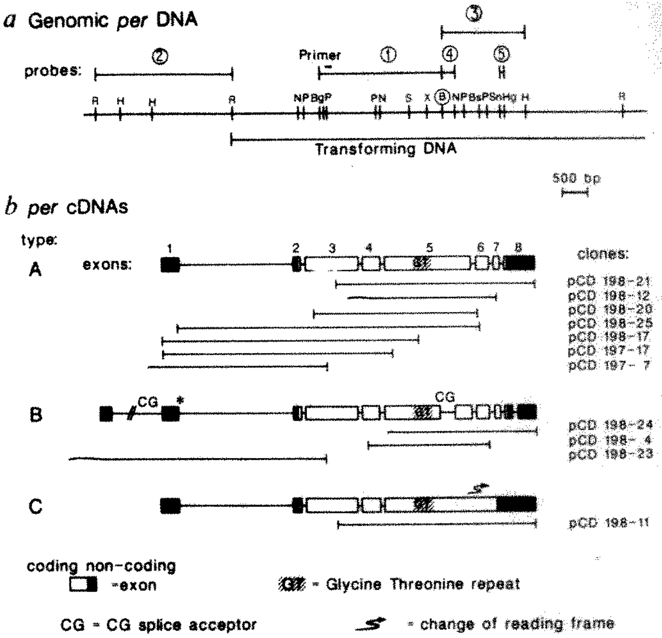

Fig. 1 Maps of *per* gene and cDNAs. *a*, Genomic restriction enzyme map of the *per* gene[6]; *b*, maps of *per* cDNAs as obtained in this study. *a*, The genomic probes used for the cDNA cloning experiments and subsequent mapping experiments are shown above the map and are referred to by numbers. A genomic fragment which was used in previous P-element mediated transformation experiments[7] is indicated below the map. Its 3′ end extends beyond the right end of the map. Its 5′ end, however, does not include the 5′ end of the transcription unit of the 4.5-kb mRNAs. The location of the specific oligonucleotide (GGAATCGGAAAGCTCTGTGC) that was used to prime cDNA synthesis from the 5′ end of the *per* mRNA is indicated by a dash. Restriction enzymes symbolized as follows: R, *Eco*RI; H, *Hin*dIII; N, *Nco*I; P, *Pst*I; Bg, *Bgl*II; S, *Sac*I; X, *Xmn*I; B, *Bam*HI; Bs, *Bst*EII; Sn, *Sna*BI; Hg, *Hgi*AI. *b*, The three proposed structures of *per* cDNAs are presented as types A, B and C. The specific cDNA clones which were analysed in detail to derive these structures are presented below the relevant cDNA maps; their names are in the column on the right. Their borders are defined by vertical bars, unless a cloning artefact such as intramolecular recombination or attachment of irrelevant sequences took place. These events occurred only at the 5′ ends of some clones and are represented by wavy lines. The cDNA maps are made of boxes (exons) or lines (introns). Open boxes, coding sequences; black boxes, non-coding sequences. The exons are numbered only in the abundant type A cDNA. CG designates the introns containing non-consensus, CG-mediated, 3′ splice sites (referred to in text), and GT the region of the glycine-threonine repeat coded for by *per* cDNAs (see text). Broken arrow indicates the change of the reading frame of type C cDNA as compared to types A and B (see text); asterisk, (type B cDNA) use of a 5′ splice junction site 20 bp upstream of the one used by type A for the splicing of exon 1 to exon 2.

Methods. RNA was prepared from heads, bodies, whole flies and embryos of *D. melanogaster* (Canton S strain), essentially as described[36–38]. All the probes used for hybridizations were DNA fragments labelled by the oligo-labelling technique[39], using random primers from Pharmacia. Probe 5 was a 65-bp *Sna*BI–*Hgi*AI fragment from clone pCD198-11 that was subcloned into the *Hin*cII and *Pst*I sites of pSP64. The labelled RNA probe was synthesized using SP6 polymerase from Promega Biotec, following their protocol. Hybridizations were carried out for 24–36 h at 42 °C in 50% formamide, 5 × SSCPE (500 mM NaCl, 75 mM Na citrate, 50 mM KH_2PO_4, 5 mM Na_2 EDTA, *p*H 7.2), 10 × Denhardt's, 0.5 mg ml^{-1} herring sperm carrier DNA. When the RNA probe was used, transfer RNA (10 μg ml^{-1}) was included in the hybridization mixture. Probes were used at 10^6 c.p.m. ml^{-1}. For cDNA cloning, 9 μg of poly$(A)^+$ RNA, made from heads of *D. melanogaster* strain Canton S, were used as described[40,41] with the following modifications. First strand was synthesized using MLV cloned reverse transcriptase (BRL, 2,000 U per reaction). The reaction was carried out in the buffer described[40], but included, in addition to oligo(dT), 200 ng of the oligonucleotide primer described above. The reaction mixture was incubated 60 min at 37 °C. Second strand synthesis was as described[40], except that no ligase was added, and the incubation was 12 h at 12–14 °C after which fresh aliquots of DNA polymerase I and RNase H were added for an additional incubation of 2 h at 22 °C. The cDNA was then blunted, *Eco*RI methylated and linkered, after which the free linkers were separated from the cDNA on a Sepharose 4B-CL column. To obtain the non-size-selected library, the cDNA was ligated to λgt11 phage arms[42], which were previously digested with *Eco*RI and dephosphorylated. Another fraction of the cDNA was electrophoresed through a 1% low-melting-temperature agarose gel and the cDNA >1.7 kb in size was extracted and ligated to λgt11 arms to obtain a size-selected library. Both libraries were packaged using Gigapack (Vector Cloning Systems) packaging extracts and were screened before amplification. Probe 1 (see above) was used to screen the size-selected library and 32 positive clones were identified. Probe 2 was used to screen the non-size-selected library and 19 clones were identified. The other probes denoted above were used to analyse and classify the isolated clones.

previous experiments using Northern blots and *in situ* hybridizations[9], namely, that *per* is mainly expressed in nervous tissue. That this type A 4.5-kb band originates from the *per* locus was confirmed by its absence from RNA of mutant per^- flies which are homozygous for a deletion of the *per* locus (lane D, Fig. 3*b*). RNA from mutant flies, transformed with wild-type genomic DNA which restores their circadian phenotype (see Fig. 1*a* for the 'transforming DNA', fragment '14.6' in ref. 7), shows the reappearance of a faint hybridizing band slightly larger than 4.5 kb (lane T, Fig. 3*b*). An additional band of low M_r is also observed in the lane with RNA from flies' heads. As it also appears in the lane with RNA from per^- flies, this band has not been characterized further.

Type C—alternative reading frame

One of the cDNA clones (pCD198-11, Fig. 1*b*), denoted type C, does not splice out the three most 3′ introns of type A. Thus it contains a very large exon (of about 2,800 bp) starting at the 5′ border of exon 5 of type A and ending at the end of exon 8. The clone extends on its 5′ side up into exon 3 where the cDNA ends. On the 5′ side of the *Bam*HI site (circled in Fig. 1*a*), this clone contains splices identical to type A (between exons 3 and 4, and 4 and 5; see Fig. 1*b*). A rough estimate is that the frequency of type C cDNA is about 10% of *per* cDNAs, as only one out of the 8 clones which extend far enough to the 3′ to be classified was of this type.

To assess the expression of type C mRNA, we have subcloned probe 5 (Fig. 1*a*), which hybridizes to type C but not type A or B DNA sequences (see below and data not shown). The use of this fragment to probe Northern blots revealed a band of ~4.5 kb that, like type A mRNA, is expressed mainly in adult flies' heads and is not present in RNA from per^- adults (see upper band in Fig. 3*a*). The band which hybridizes to the type C-specific probe is slightly larger than the type A band (compare to Fig. 3*b*, which is the same blot reprobed with type 'A+C'-specific probe). The small difference in size can be accounted for by the three type A introns, 193 bp altogether, which are not excised in type C. The small size difference also indicates that the 2.3-kb intron (between exons 1 and 2 of type A) cannot be part of the type C mRNA. We assume therefore that the 5′ end of this mRNA is identical to that of type A.

Type C cDNA diverges from type A at the end of exon 5 (Figs 1*b* and 2). Interestingly, the sequence of the last 107 amino acids of the putative type C protein is entirely different from the 149 amino acids at the end of the type A protein. This stems from the fact that the type C mRNA, by reading through the intron which lies between exons 5 and 6 of type A, then reads through the entire exon 6 in a different frame from the one used by type A (Fig. 2).

Type B mRNA uses a non-consensus splice site

Of the 16 cDNA clones that span the *Bam*HI site (circled in Fig. 1*a*), two clones (~10%) did not have this site (pCD198-4 and pCD198-24, Fig. 1*b*). One of the clones (pCD198-24) ends at the same 3′ region as do types A and C. But in this clone exon 8 of type A is split into two exons by an 89-bp intron (see dashed underlines indicating the splice junctions of this intron in the bottom of Fig. 2). This splice does not result in any changes in the putative protein coding sequence, because it occurs downstream of the stop codon shared by type A and B cDNAs. The *Bam*HI site is missing from these 2 clones due to yet another splicing event which takes place in exon 5 of type A. In this instance a relatively large intron of 288 bp is excised, resulting in a deletion of 96 amino acids from the predicted type A protein sequence (see Fig. 1*b* for the CG intron; see Fig. 2 for the predicted type B protein sequence). The 3′ splice site

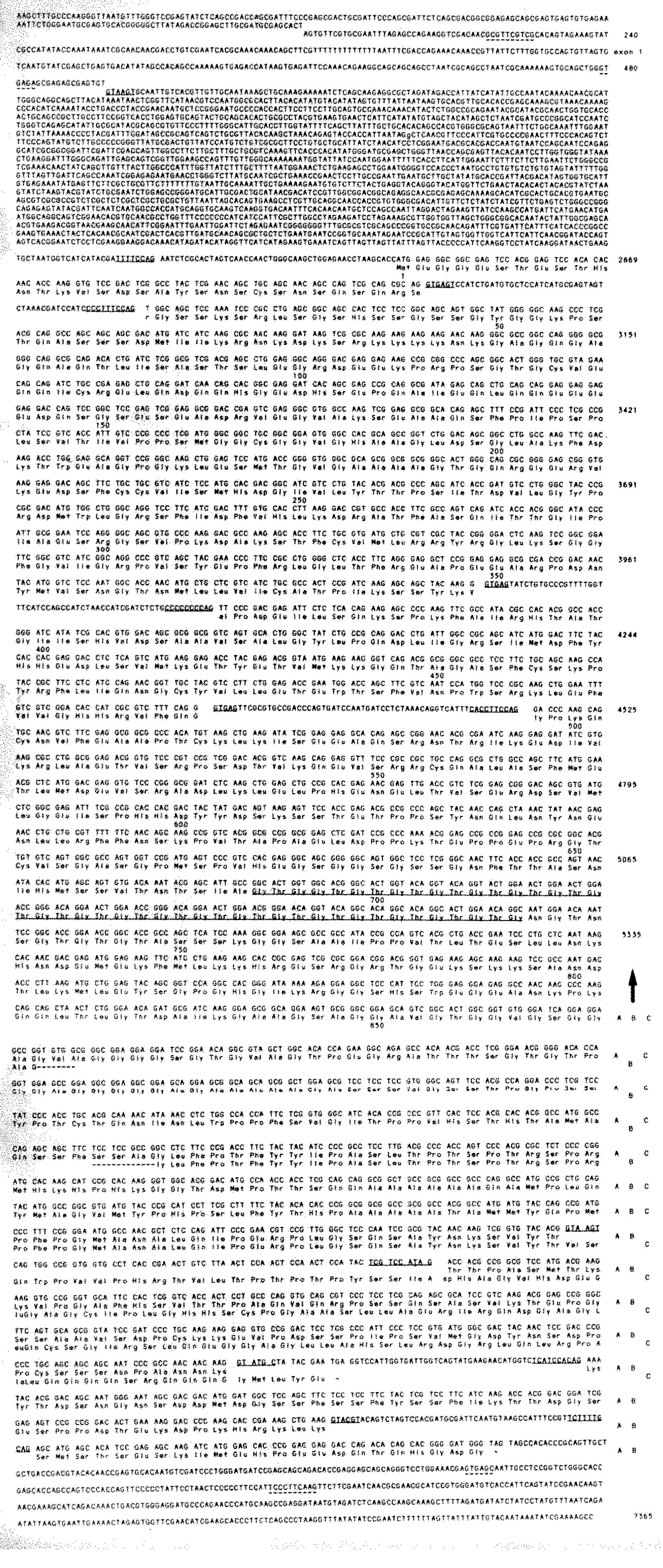

Fig. 2 DNA sequence of the *per* gene and the predicted amino acid sequence of the putative *per* proteins. The sequence of genomic *per* DNA from *D. melanogaster* (Oregon R strain) was obtained on both strands by the dideoxy method as described.[47,48] The cDNA clones were mapped by comparison to the genomic DNA; in places where they differed, the cDNAs were sequenced according to Maxam and Gilbert[43]. In this way the precise positions of introns were determined; these positions were consistent with those obtained by RNase protection experiments (data not shown). The protein sequences are derived from the conceptual translation of the relevant sequences. Exon 1 is non-coding, and the predicted initiator methionine is present in exon 2. This methionine is followed by a very long open reading frame (in the derived cDNA sequences) and is preceded by an in-frame stop codon. Its surrounding sequences conform well to rules established for translational start sites[44], especially for *D. melanogaster* (D. Cavener, personal communication). The numbers within the sequence indicate amino acids. The numbers in the right margin indicate nucleotides from the *Hin*dIII site, which is 178 bp upstream of the beginning of exon 1 (the second H from the left in Fig. 1*a*). Exon 1 is non-translated and is set off from the upstream nontranscribed sequence as well as from the downstream large intron (intron 1) by shifts in the printed lines. The transcription start is at position 179, as determined by RNase protection mapping (data not shown and ref. 10). In addition, two cDNA clones, pCD198-17 and pCD197-17, extended to positions 192 and 210, respectively. The alternative 5′ junction, which is used by clone pCD198-23 (presumably type B cDNA) to join exon 1 to exon 2, is indicated by a broken underline. The possible 3′ splice junction of this clone near the 5′ end of exon 1 is also indicated by a broken underline, as are all the additional type B-specific splice junctions throughout the sequence. The rest of the splice junctions are shared by type A and B cDNAs and are underlined. All three putative proteins share the first 862 amino acids of the protein sequence. The protein sequences of the three proteins (*per* A, B and C) are indicated by the letters in the right margin which designate which of the *per* proteins share the sequence in the line to the left. As indicated in the text, type C cDNA does not splice out the last three introns of type A cDNA; the resulting alternative reading frame used by type C is shown in the line below the translation of the open reading frame used by types A and B for this sequence. The first letter in the three letter amino acid code is written below the first base of the translated codon triplet. The 3′ untranslated sequence present in the cDNA clones is also shown up to the point of poly(A) tail addition. A comparison of the genomic sequence of Oregon R *per* DNA shown with ~4,800 nucleotides of *per* DNA from Canton S[10] reveals only 14 base substitutions. Nine of these are in the coding DNA and all nine are third base isocoding substitutions. Four are in the introns (one in intron 6 and three in intron 7) and one is in the 3′ untranslated region. The Canton S sequence[10] lacks the C at position 5,290 in the Oregon R sequence. Also, the Thr-Gly repeat region is 18 nucleotides (or six pairs of Thr-Gly) longer in Canton S[10] than Oregon R; this is a true strain polymorphism and will be the subject of a separate report.

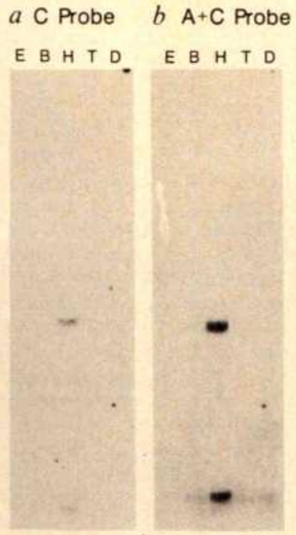

Fig. 3 Northern blot analysis of type A and C mRNAs. 10 μg of poly(A)$^+$ RNA from embryos (E), bodies (B), heads (H), total flies transformed with *per* genomic DNA (see Fig. 1*a* for 'transforming DNA') (T), and total flies deficient for *per* DNA, *Df(1)TEM202/Df(1)64j4* (ref. 6) (D), were electrophoresed through a 1% agarose formaldehyde gel and transferred to a membrane filter. (Note that electrophoresis was carried out for a relatively long time to resolve type A and C mRNAs.) *a*, Blot hybridized first to a type C-specific RNA probe (see probe 5, Fig. 1*a*), then washed (see Fig. 1) and exposed to X-ray film for 4 days. *b*, The same blot rehybridized to the *Bam*HI-*Nco*I DNA fragment (Fig. 1*a*, probe 4), which hybridizes to both type A and C cDNAs but not to type B. The blot was exposed to film for 24 h. The previous type C-specific signal, which was much fainter than the present one, washed off during the rehybridization.

	5′ Junction	3′ Junction
Consensus:	G/GT$\genfrac{}{}{0pt}{}{A}{G}$AGT	(pY)N$\genfrac{}{}{0pt}{}{C}{T}\underline{A}$G/
pCD198-24 & 4:	G/GTGTGG	CTTCTCCTCCGC$\underline{C}$G/
pCD198-23:	?	CAACGCGTTCGT$\underline{C}$G/

Fig. 4 Non-consensus splice sites in type B cDNA. The 5′ and 3′ splice site sequences of the CG-mediated splice of type B cDNA (clones pCD198-4 and pCD198-24) are shown below the consensus 5′ and 3′ splice site sequences[45]. The possible CG-mediated 3′ splice site at the 5′ end of type B is also shown (clone pCD198-23). As discussed in the text, the 5′ splice site of this putative intron is not known; (pY) denotes a polypyrimidine stretch.

in this case deviates from the AG rules[13], using the dinucleotide CG instead. The sequence immediately 5′ to the splice site is rich in pyrimidines and agrees well with the rest of the consensus for 3′ splice sites (see Fig. 4). The 5′ splice site obeys the consensus rules reasonably well (Fig. 4). The CG splice site is present in both Canton S[10] and Oregon R (this study) genomic sequences. Moreover it is present in the DNA sequence of the type A and C cDNAs (which do not use this splice junction). It is also noteworthy that *D. melanogaster* has only one *per* gene from which type B mRNA must be transcribed.

We found evidence in yet a third clone (pCD198-23, Fig. 1*b*) for a similar event occurring in a different position. In this clone, a new sequence is joined to a point 47 bp downstream of the transcription initiation site of exon 1 of type A (see dashed underline at the 5′ end of exon 1 in Fig. 2). As we have not analysed the 5′ side of this putative intron, we cannot be certain that it is a true splicing event, that is, one using a consensus 5′ splice site. It is interesting, however, that the putative 3′ splice site is again a CG rather than an AG dinucleotide. Figure 4 shows the 12 nucleotides immediately 5′ to the two non-consensus 3′ splice junctions described above. In both cases the pentamer CGYCG (with Y denoting a pyrimidine) precedes the 3′ junction.

The splicing event between exons 1 and 2 is also slightly different in clone pCD198-23, where the 5′ splice site of exon 1 is 20 bp 5′ to that used by the abundant type A (see dashed underline near the 3′ end of exon 1 in Fig. 2). It is noteworthy that both of the changes in exon 1 of clone pCD198-23 do not result in protein sequence changes. The putative extra 5′ exon of this clone, however, does suggest that the mRNA complementary to it may use a different promoter, one which is 5′ to that used by type A and presumably by type C. The fact that a CG 3′ splice site may be used in two different positions in two independent and relatively rare *per* cDNA clones raises the possibility that they might originate from the same mRNA. Therefore, in spite of the fact that clones pCD198-24 and pCD198-23 are not linked, we have classified both of them as type B cDNAs (Fig. 1*b*). But further analysis will be necessary to clarify the relationship of clone pCD198-23 to downstream sequences.

We have tried to assess the expression of type B mRNA and the occurrence of the unusual, CG-mediated splice by RNase protection experiments. We did observe protected bands which are consistent with the unique splices of type B mRNA (not shown); but because of the low abundance of this transcript, other equally intense background bands obscured the results. A Northern blot analysis with a type B-specific probe was not possible because all type B sequences are included in types A and C (Fig. 1*b*); also, the putative 5′-specific sequence of clone pCD198-23 was not separable from sequences shared with type A.

Unusual splicing events

The use of an alternative reading frame, similar to that described here for type C cDNA, as a means of increasing the coding potential of a gene is unusual in eukaryotes, probably because it renders the gene more sensitive to the effects of mutations. Such a situation has been described for some bacterial as well as mammalian viruses[14,15], where the rationale would be the need to compact the genetic material. The only other reported evidence for a similar event in eukaryotes (in the case of the human gastrin-releasing peptide prohormone mRNAs[16]) is incomplete because the corresponding genomic sequence is not known.

Although deviations from the 5′ splice junction 'GT rule' exist (there are a few reported cases of a GC 5′ splice site[17–21]), the 3′ splice site 'AG rule' is strictly conserved[13]. To the best of our knowledge, the case described here for type B *per* cDNA is the first to deviate from the AG 3′ splice site rule[13]. The presence of a pyrimidine in the −2 position (C instead of A) is especially noteworthy. One possibility to explain this finding is that some novel aspect of the splicing machinery—perhaps associated with the brain or a specific subset thereof—may be necessary to recognize this unusual splice site. Were the second case of a 3′ CG junction (in clone pCD198-23) shown to be genuine, the argument for such a novel mechanism would be further strengthened. Consistent with such a mechanism is the fact that three of the four unusual cDNAs show more than one deviation from the major type A structure.

Intron recruitment

It has been proposed by Gilbert[22] that a single point mutation at a splice junction can contribute to gene evolution by recruiting a whole intron to become part of a coding exon. Perhaps the unusual CG 3′ splice site used by type B cDNA was once a consensus and efficient AG 3′ splice site preceded by a polypyrimidine stretch (Fig. 4). According to this evolutionary scheme, the 'historically' abundant *per* transcript might have

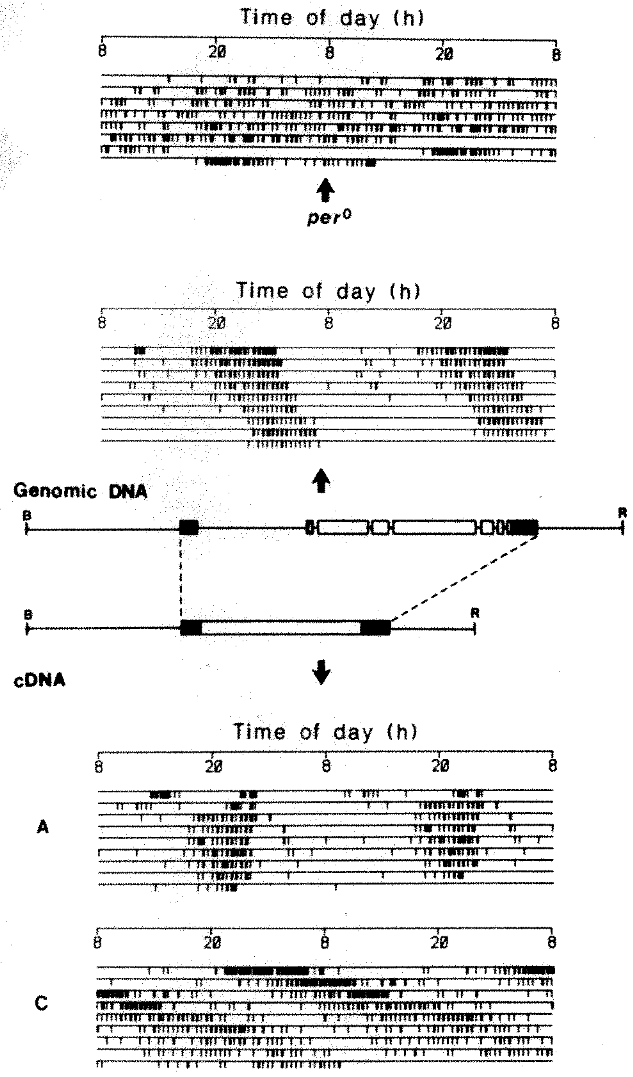

Fig. 5 Restoration of circadian rhythms to *per*^{o} flies by transformation with the genomic '13.2' fragment and with type A and type C cDNAs. The type A cDNA construct was tailored from clones pCD198-21 and pCD198-25 and type C cDNA was constructed from clones pCD198-11 and pCD198-25, which were ligated to flanking genomic sequences through a unique *Mlu*I site in exon I and the downstream *Hin*dIII site in exon 8. Type A and C cDNAs (see Fig. 1*b*), and the genomic DNA which codes for them, were ligated into P-element transformation vectors[46] such that they were flanked by 3.7 kb of 5′ genomic sequence (up to the 5′ *Bam*HI site, see ref. 6) and by 2.0 kb of genomic sequence at the 3′ end (down to the *Eco*RI site; see Fig. 1*a*). These vectors included the *rosy*$^{+}$ marker[46]. Transformants (whose genetic background consisted of *per*^{o} and *ry*^{506}) were screened for a *ry*$^{+}$ phenotype (see ref. 35). Adults from these strains, carrying one dose of a given 'insert'-bearing chromosome (again marked by *ry*$^{+}$), were monitored for locomotor activity under free-running conditions[35], and the accompanying circadian rhythmicity (or lack of it) was assessed (see below). The activity records shown are for one control *per*^{o} (*ry*^{506}) segregant from strain '13.2:2' (see Table 1) in the top panel; for a fly transformed with the 13.2 kb of genomic *per* DNA (from strain '13.2:2') in the second panel; for a type A cDNA transformant (strain 'cA:22') in the third panel; and for a type C cDNA transformant (strain 'cC:46') in the bottom panel. The period estimated for the '13.2' fly (from χ^2 periodogram analysis[35]) was 24.5 h, that for the type A cDNA fly was 24.0 h and that for type C was 28.5 h. The *per*^{o} record shown here was statistically arrhythmic. The 13.2-kb genomic fragment is identical to the 12.8-kb fragment used in a previous experiment[35]. We presume that the phenotypic rescue was less successful in these previous experiments for artefactual reasons.

been type B mRNA. A splice junction point mutation (see, for example, ref. 23) could have occurred to change the AG to CG, resulting in a reduction in the efficiency of this 3′ splice site (surprisingly not to zero) and a recruitment of the coding potential of the type B intron (labelled as the downstream 'CG' in Fig. 1*b*) to the new type A *per* mRNA.

Because *per* is a gene that controls complex behavioural phenotypes, it may have undergone more recent evolutionary changes than many other genes; the unusual type B and C mRNAs may then reflect recent events in the history of *per*. Perhaps there has not been sufficient time for further evolution to eliminate or to stabilize these unusual transcripts. It is noteworthy, in this regard, that the mRNAs of the *Drosophila* memory gene *dunce* show marked heterogeneity[24].

Types A and C rescue arrhythmic flies

Previous germ-line transformation experiments of mutant arrhythmic (*per*^{o}) flies, using genomic DNA from the *per* region, have indicated that this DNA is indeed involved in the biological clock phenotype. But our data (Figs 1 and 2) and those of Jackson *et al.*[10] indicate that none of the genomic fragments used in the early transformation experiments include the entire transcribed sequences of any of the three 4.5-kb transcripts described here. The absence of part of the 4.5-kb transcription unit may explain the incomplete phenotypic rescue obtained in previous studies[7,8].

To examine the involvement of the 4.5-kb transcripts in the control of clock functions we performed two kinds of transformation experiments. (1) Transformation of *per*^{o} arrhythmic flies with a 13.2-kb genomic DNA, which (we now know) contains the entire transcription unit for, at least, type A and C mRNAs. In addition, this construct contains 3.7 kb of 5′ flanking sequences and 2.0 kb of 3′ flanking sequences. (2) Transformation of arrhythmic flies with a construct containing type A cDNA and with a construct containing type C cDNA, each containing the same flanking sequences as described above for the genomic fragment. Because we do not as yet have the 5′ sequences of type B cDNA, we could not carry out transformation experiments with this cDNA.

Figure 5 shows typical activity profiles (actograms). A *per*^{o} control fly (upper panel) shows scattered bursts of activity with no pattern and was arrhythmic by periodogram analysis. A *per*^{o} fly transformed with the 13.2-kb genomic DNA fragment (second panel) shows circadian rhythmicity that is as strong as that of flies with endogenous wild-type *per* DNA. The penetrance (the fraction of flies that show the phenotype) of these transformants is also very high (see Table 1). This indicates that the 13.2-kb genomic fragment may contain all the coding and regulatory sequences needed for a full restoration of the clock function. The type A cDNA transformant (third panel) also shows restoration of circadian rhythms similar to that of the 13.2-kb genomic transformant and of wild-type flies. The penetrance of type A transformants, however, is lower than that of the genomic transformants (Table 1). The lower panel of Fig. 5 shows the actogram of a type C cDNA transformant. This fly also showed a rhythmic phenotype, though its period is longer

Table 1 Transformants carrying genomic DNA or cDNAs from *per*

Transforming DNA	Transformants	No. rhythmic	Mean period (h ± s.e.m.)	No. arrhythmic
Genomic				
13.2 kb	13.2:2	17	24.9 ± 0.1	0
	13.2:34	21	24.7 ± 0.1	2
cDNA				
type A	cA:8	2	24.3 ± 1.3	4
	cA:22	9	23.8 ± 0.3	3
type C	cC:32	0	—	3
	cC:46	4	26.8 ± 0.8	3
	cC:55	0	—	2
	cC:68	1	24.5	6
	cC:74	1	25.0	2
Controls				
	per^{+}/*per*^{o}	11	24.6 ± 0.2	0
	per^{o}	0	—	12

The 13.2-kb genomic DNA and type A and type C cDNA constructions were generated as described in Fig. 5 and used to produce germ-line transformants. Two independently isolated strains for both the genomic 13.2-kb construct and for the type A cDNA construct, and five for the type C cDNA construct, were isolated and tested for rhythmicity (see Fig. 5). The transformed strains are named in the second column, with arbitrary isolation numbers following the colons. Summaries of the behavioural results are listed in the three other columns. The proportions of significantly rhythmic adults and mean circadian periods (±s.e.m.) from each strain were derived based on the testing and analytical methods cited in Fig. 5. Each transformant carried one 'dose' of a *per* DNA insert-containing autosome. Males from the two '13.2' strains yielded shorter periods (24.5 ± 0.1 h, $n = 23$) than did females (25.1 ± 0.1 h, $n = 15$); this implies that the gene's activity in these transformants is dosage compensated[33], such that one 'dose' of *per*$^{+}$ in a male is expressed at a higher level (leading to a shorter period, see below and ref. 34) than the equivalent dosage in a female, even when this X-chromosomal material is transduced to an autosome (see ref. 33) in the '13.2' strains. Controls were thoroughly *per*^{o} in genotype, or carried one dose of normal *per* information on one of their X chromosomes (the 11 *per*^{o} heterozygotes). The latter genotype routinely leads to periods slightly longer than normal values (which are 24.0 ± 0.1 h, from tests of 23 *per*$^{+}$; *ry*^{506} males; refs 34, 35).

than normal as indicated by the slope of the actogram. The penetrance of type C transformants (Table I) is also lower than that of the genomic 13.2 kb transformants. This type C cDNA transformation experiment should be interpreted with caution because type C cDNA could potentially give rise to type A mRNA by further splicing events (see Fig. 1*b*).

In contrast to the genomic 13.2-kb fragment, the inability of the cDNAs to restore a wild-type-like circadian phenotype, with a period of about one day and essentially all individuals being rhythmic, may be due to one of the following. (1) The absence of regulatory sequences, which may be present in the missing introns (especially the large first intron), may affect the tissue distribution and levels of expression of the mRNAs. (2) Splicing *per se* may affect the level of mRNA transport to the cytoplasm[13]; thus transformation with the genomic construct may result in higher steady-state levels of mRNA. (3) Unlike the cDNAs, the genomic fragment may have the potential to encode all of the *per* proteins, two or more of which may be necessary to restore a full circadian phenotype. Additional transformation experiments and mRNA expression studies will be needed to sort out these possibilities.

The *per* proteins

The three cDNA types cloned in this study potentially code for three proteins. Our transformation experiments indicate that at least *per* A and possibly *per* C are involved in clock function. Recent work has molecularly mapped the *per*^{o} and *per*^{s} mutations to type A's exon 4 and the 5′ half of exon 5, respectively (ref. 25 and M. W. Young, personal communication). Because these mutations lie in sequences that are shared by the three cDNAs (Figs 1 and 2) and may affect the function of all three *per* proteins, they do not indicate whether the putative proteins *per* B and C are also clock protein(s).

An attractive possibility is that one *per* protein (probably *per* A) functions in the circadian clock, whereas another one functions in the courtship song clock[4]; a third may function in yet another clock phenotype (see, for example, ref. 26). This possibility is especially interesting because different species of *Drosophila*, although they exhibit the same 24-hr circadian period, differ markedly in their courtship song period length[3,4,27], indicating a possible divergence in the control of the two clock phenotypes. Moreover, mosaic analysis of *per* mutations has indicated that the circadian clock is located in the fly's head[28], whereas the courtship song clock seems to reside in the fly's body, probably in the thoracic ganglia[3]. It will be interesting to see whether the precise spatial distribution of type B (as well as type C) mRNA is different from that of type A.

Because the three *per* proteins inferred here differ in only about 10% of their sequence from each other (see Figs 1 and 2), it seems reasonable to assume that they share the proteoglycan properties, especially since they all contain the entire Gly-Thr amino acid repeat[8,10,11]. Proteoglycans have been shown to be involved in many aspects of vertebrate nervous systems[29-32]. Perhaps the multiplicity of putative *per* proteins, described here, indicates that *per* products are not confined to clock phenotypes, but may also participate in other nervous system functions.

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Cosmic bubbles as remnants from inflation

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It is generally assumed that primordial density perturbations necessary for galaxy formation are adiabatic perturbations with a flat (scale-independent) spectrum formed during inflation[1-4]. It is not clear, however, whether it is possible to create all types of large-scale structures in the Universe (quasars, galaxies, clusters, superclusters, for example) from one type of perturbation with such a simple spectrum. An alternative approach is based on the theory of cosmic strings[5,6]. However, it is difficult to reconcile this approach with the inflationary paradigm[7]. Recently it was shown that inflation may produce not only adiabatic perturbations but also isothermal perturbations[7-10] with the spectra considerably different from the flat spectrum[7,8,10]. Moreover, phase transitions which occur at the last stages of inflation may lead to formation of exponentially large bubbles and domains containing matter with different density[7]. Here we demonstrate that such effects may be responsible for the observed foam-like large-scale structure of the Universe[11].

Most contemporary theories of galaxy formation adopt the modern cosmological paradigm, which includes three successive stages: the very early inflationary stage ($t < 10^{-35}$ s), producing a flat Universe, the density parameter, $\Omega_{\rm tot} = 1$, the hot radiation-dominated Friedman stage, and the matter-dominated stage. As a result of gravitational instability during the last stage, the large-scale structure of the Universe arises from small density perturbations of matter.

Observations performed during the past few years demonstrate that galaxies and clusters of galaxies are not distributed randomly but form systems of different scale, the space between these systems being void of galaxies[12,13].

A recent deep survey of galaxies in a 6° × 114° strip shows the present of bubbles of diameter 30–100 H^{-1} Mpc (H is the Hubble constant in units of 50 km s^{-1} Mpc^{-1}), completely void of galaxies and having sharp boundaries[11].

In the following we shall define two phases in space, bubbles constitute phase B, and the space between bubbles phase S (see also ref. 14). All observed structures are located in phase S, but still the space is largely void of galaxies[13]. Galaxies in phase S form systems of at least three scales[13]: groups of scale $\sim 1\,H^{-1}$ Mpc, clusters of galaxies and galaxy chains of scale $\geqslant 10\,H^{-1}$ Mpc and superclusters (and other inhomogeneities) of scale 50–200 H^{-1} Mpc. Observations of the Local Supercluster demonstrate the absence of both bright and faint galaxies in voids within and around superclusters. The available data suggest that bubbles are also void of dwarf galaxies[14]. In total one can distinguish structures of several types, supercluster-void structures of small, medium and large scales in phase S, and voids in phase B.

Numerical experiments[15,16] with biased galaxy formation demonstrate that supercluster-void structure of small and medium scale (phase S) can be reproduced in a cold dark matter dominated Universe, however, they are unable to explain the presence of empty bubbles with their relatively sharp boundaries (phase B). The fine structure in phase S is not reproduced in the hot dark-matter-dominated Universe. Thus the presence of structure of different scales and large empty bubbles is a serious challenge to theories of galaxy formation. In what follows we will suggest some possible ways to solve this problem in the context of inflationary cosmology (many relevant details can be found also in ref. 7).

In our opinion, the most natural version of the inflationary universe model is the chaotic one[17]. As distinct from the new inflationary universe scenario, the chaotic inflation model can be realized in a wide class of theories of elementary particles under rather natural initial conditions in the very early universe[18-20]. According to the simplest version of this model, inflation is driven by some massive weakly interacting scalar (inflation) field ϕ. During inflation this field slowly decreases from some initial value $\phi \geqslant M_{\rm P}$ where $M_{\rm P} = 1.2 \times 10^{19}$ GeV, whereas the universe at the stage of inflation expands $\exp[(2\pi/M_{\rm P}^2)\phi^2]$ times[17]. Inflation stops when the field ϕ becomes smaller than $O(\frac{1}{3}M_{\rm P})$. Large-scale quantum fluctuations of this field lead to adiabatic perturbations of density with an almost scale-independent spectrum[1-4].

It was widely believed that such perturbations could be generated only during inflation. This is indeed the case in the theories which describe the evolution of the inflation field only[1-4]. However, in realistic theories of elementary particles there exist many other types of scalar fields $\psi_{\rm i}$. Their perturbations, which are also generated during inflation, in some cases lead to isothermal perturbations of a considerable amplitude[7-10]. What is more important is that the spectrum of such perturbations may differ from the flat one, for example, the spectrum may decrease at small (at large) wavelengths[7,8,10] and may have several sharp maxima[7].

Especially interesting effects may arise due to phase transitions which occur at the last stages of inflation[7]. In the model discussed above, perturbations on a scale of horizon ($l_h \sim 10^{28}$ cm) are produced when the field ϕ reduces to the value $\phi_h \sim 3.2\ \mathrm{Mp} \sim 4 \times 10^{19}$ GeV. Perturbations on the galaxy scale ($l_{\rm g} \sim 10^{22}$ cm) are produced at $\phi \approx \phi_{\rm g} = 2.8\ M_{\rm P}$. Let us assume that the field ϕ interacts with the fields $\psi_{\rm i}$, that is, $\Delta \mathrm{L}(\phi, \psi_{\rm i}) = -\lambda_{\rm i}\phi^2\psi_{\rm i}^2$. Typical values of $\lambda_{\rm i}$ should not be too large, $\lambda_{\rm i} \leqslant 10^{-6}$. When the field ϕ decreases from ϕ_h to $\phi_{\rm g}$ the effective mass squared of the fields $\psi_{\rm i}$ is changed approximately by 20%, $\Delta M_{\rm i}^2 \sim 2\lambda_{\rm i}(\phi_h^2 - \phi_{\rm g}^2) \sim \lambda_{\rm i}(2 \times 10^{19}\ \mathrm{GeV})^2 \lesssim (2 \times 10^{16}\ \mathrm{GeV})^2$. But this is the scale of grand unification. Such a change of $\Delta M_{\rm i}^2$ may lead to phase transitions with generation (or disappearance) of large classical fields $\psi_{\rm i}$ (ref. 7). Such phase transitions are completely analogous to the well-known high-temperature phase transitions in gauge theories[21,22].

Therefore in realistic theories, in which there are very many different types of the fields $\psi_{\rm i}$, one may expect a sequence of different phase transitions which occur at some critical values of the field $\phi = \phi_{\rm i}$ when this field decreases from ϕ_h to $\phi_{\rm g}$. If some of these phases transitions are the first order ones, they proceed by formation of bubbles of the fields $\psi_{\rm i}$. The energy density of all fields $\psi_{\rm i}$ during inflation remains much smaller than the energy of the inflation field ϕ. Therefore the interior of each bubble continues expanding exponentially, just as before the phase transition. As a result, at the end of inflation the size of each bubble $l_{\rm B_i}$ grows

$$\exp\left(\frac{2\pi}{M_{\rm P}^2}\phi_{\rm i}^2\right)$$

times. A typical size of each bubble $l_{\rm B_i}$ after inflation is given by

$$O(M_{\rm P}^{-1})\exp\left(\frac{2\pi}{M_{\rm P}^2}\phi_{\rm i}^2\right)$$

and for $\phi_{\rm i}$ in the range $\phi_{\rm g} \leqslant \phi_{\rm i} \leqslant \phi_h$ the typical bubble size lies in the region $l_{\rm g} \leqslant l_{\rm B} \leqslant l_h$. This may give rise to the bubble-like large-scale structure of the universe discussed above[3].

Note, that if there is only one first-order phase transition during the evolution of the field ϕ from ϕ_h to $\phi_{\rm g}$, then all large-scale bubbles which can be observed now have a single typical scale $l_{\rm B}$, which is

$$\left[\frac{2\pi}{M_{\rm P}^2}(\phi_{\rm i}^2 - \phi_h^2)\right]$$

times smaller than the horizon scale $l_h \sim 10^{28}$ cm. However, if many such transitions occur at different values of ϕ_i, the hierarchy of scales appears,

$$l_{B_i}/l_{B_j} \sim \exp\left[\frac{2\pi}{M_p^2}(\phi_i^2-\phi_j^2)\right].$$

In a more general case, phase transitions during inflation lead to formation of exponentially large domains (but not exact bubbles) containing matter in different phases. For example if the field ψ is the Higgs field responsible for the SU (5) symmetry breaking in grand unified theories the universe at an intermediate stage of its evolution may become divided into domains with symmetry breaking $SU(3)\times SU(2)\times U(1)$ or $SU(4)\times U(1)$. Later $SU(4)\times U(1)$ phase becomes unstable and also returns to the phase $SU(3)\times SU(2)\times U(1)$. The behaviour and structure of domains is crucially model dependent. For example baryon density produced inside different domains after inflation may differ by several orders of magnitude. Domains with a smaller free energy density of matter will expand and if they are relatively rare, they become more and more like bubbles (phase B). On the other hand, if the domains of the energetically unfavourable phase are sufficiently rare, they look like exponentially large drops with a gradually decreasing size. The domain contraction is followed by an explosion and formation of a more or less spherical shell of dense matter rapidly moving from the centre of explosion. This process resembles that suggested by Ostriker and Cowie[23], but it has a different physical origin. In particular, the domain contraction and explosion may occur much earlier than the time of recombination, and the shell may consist of elementary particles of all types rather than just of baryons.

The two-phase structure of the universe may emerge also due to isothermal perturbations generated at the moment of the second-order phase transition during inflation. Such perturbations have a spectrum with a sharp maximum displaced at some exponentially large wavelength[7]. If the relative amplitude of perturbations of the field ψ with this wavelength is sufficiently large ($\delta\psi/\psi \sim 0(1)$) then it can be shown that the corresponding density perturbations in the universe also have the foam-like structure consisting of bubbles of exponentially large size. In this case, the boundary between the B and S phases might be not very sharp.

Thus, there exist many different possibilities to obtain the observed foam-like structure of the universe due to the phase transitions during inflation. We wish to note that different mechanisms may lead to a somewhat different structure of phases B and S. In some versions of our model baryonic matter is created predominantely in phase S. Density perturbations may exist both in phases B and S, but only in phase S do baryonic matter form luminous objects. Another possibility is that baryonic matter is present everywhere, but dark matter is present only in phase S, and for this reason, density perturbations in phase B are insufficient for galaxy formation. A similar result is true if galaxy formation occurs due to isothermal perturbations of the field ψ, which may be absent inside bubbles, that is, inside phase B. A detailed observational study of the large-scale structure of the universe may show which of these possibilities (if any) is actually realized. This would be important not only for cosmology, but also for the investigation of the phase structure of the underlying theory of elementary particles.

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Detection of strong methanol masers towards galactic H II regions

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Recently, the number of transitions as well as the number of sources showing maser emission of interstellar methanol (CH_3OH) has been increased significantly. Wilson *et al.*[1,2] detected two new K-band lines to be masing towards W3(OH) and possibly NGC7538. Morimoto *et al*[3] found masers at mm-wavelengths towards Sgr B2, and Menten *et al.*[4] demonstrated that maser action in the J_2–J_1 series of E-type methanol, first observed towards Orion-KL[5], is a common phenomenon in numerous molecular clouds. Observations of the 2_0–3_{-1}E transition of methanol have led to the discovery of strong maser action in a number of galactic sources. Towards W3(OH), NGC6334 and NGC7538, these masers have flux densities of order 1,000 Jy and seem to be closely correlated with compact H II regions. The line intensities are comparable to those observed in OH. Towards other sources absorption or a combination of maser emission and absorption is observed.

The observations were carried out in July 1985 and April–May 1986 with the National Radio Astronomy Observatory (NRAO) 140-foot telescope in Green Bank. In July 1985, the data were acquired using a double-beam-switching technique. Total power mode or frequency switching was used during the 1986 observations. System temperatures were typically ~60 K. The beamwidth of the telescope is 2.3 arc min at the observing frequency of 12,178.593 MHz. The flux density scale was established by observations of NGC7027, for which a flux density of 6.33 Jy is interpolated using the formula of Baars *et al.*[6]. The typical r.m.s. pointing accuracy is estimated to be 15 arc s. The observations were greatly hindered by interference from communications satellites which operate at this frequency, and weak features were therefore carefully checked for reality.

Table 1 gives a summary of our observations: the 2_0–3_{-1} E line was detected in absorption and/or maser emission towards all but one of the H II region molecular cloud sources observed by us. In some cases (for example, Ori-KL) we observe weak broad lines. Most spectacular are the powerful masers observed towards W3(OH), NGC6334 and NGC7538 (Figs 1–3). Line parameters for these sources are presented in Table 2. From our single dish offset measurements we can put upper limits of 1.2 arc min on the sizes of the 12.1 GHz emission regions, corresponding to lower limits for the line brightness temperature of 1,200 K (W3(OH)), 340 K (NGC7538), and 2,000 K

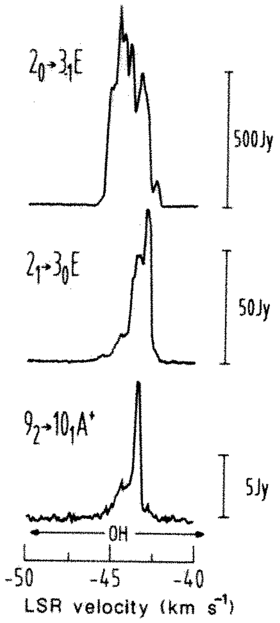

Fig. 1 Methanol masers observed towards W3(OH). The 2_0-3_{-1}E line (velocity resolution: 0.03 km s^{-1}) is shown in comparison to the 2_1-3_0E and 9_2-10_1A$^+$ lines[1,2] (top to bottom). Note that the emission covers in each case the same velocity interval. The velocity range showing OH maser emission[16] is also indicated.

(NGC6334). Figure 4 shows spectra taken towards W51 as an example of a source where a combination of maser emission and absorption is observed and towards S140 showing pure absorption. From our offset measurements, we find that the absorption towards W51 is clearly extended relative to our beam and appears at higher velocities in the western part of the source, thus showing the same velocity structure as the thermal methanol emission seen in the J_2-J_1 E lines[4]. Unlike W51, S140 has only very weak continuum emission at 12 GHz. It is therefore likely (also in the case of L183) that we are observing here.absorption of the 2.7 K microwave background radiation.

Due to its complex level structure, methanol can have a large number of transitions inverted under interstellar conditions. Experience with the observed K-band lines leads to the conclusion that the sources with cm-wavelength methanol masers observed up to now can be divided into (at least) two categories. Firstly, there are the sources (class A) which show maser emission in the J_2-J_1 ($J = 2, 3, \ldots$) series of E-type methanol. These masers can be found at large offsets (up to 2 pc) from OH and H_2O masers as well as from compact H II regions and infrared sources which makes it difficult to assign conclusively a powering mechanism to them. Additionally, and perhaps associated with class A masers, broad thermal emission is observed which comes from the compact molecular cores in these regions. Examples include Orion, W31C, W51 and DR21[4].

Table 1 Summary of measurements

Source	Right ascension α(1950)	Declination δ (1950)	V_{LSR}* (km s^{-1})	Emission or absorption
†W3(Cont)	02h 21min 56.6s	61° 52′ 30″	−45	A?
†‡W3(OH)	02 23 17.0	61 38 53	−45	M
TMC-1	04 38 38.5	25 35 45	6	—
Ori-KL	05 32 46.7	−05 24 21	9	E
§NGC2024	05 39 11.4	−01 56 09	11	?
‖S255	06 09 58.1	18 00 16	17	A?
S269	06 11 46.5	13 50 39	19	M
IRC+10216	09 45 14.8	13 30 39	−26	—
§Cen A	13 22 31.5	−42 45 26	546	?
L 183	15 51 33.8	−02 40 30	2	A?
†¶NGC6334 A	17 16 57.8	−35 51 45	−11	A
¶NGC6334 B	17 17 00.5	−35 49 49	−11	—
¶NGC6334 C	17 17 11.3	−35 48 25	−11	—
¶NGC6334 D	17 17 23.0	−35 46 20	−11	—
¶NGC6334 E	17 17 29.8	−35 43 08	−11	M
†¶NGC6334 F	17 17 32.3	−35 44 04	−11	M
Sgr A	17 42 27.8	−29 03 59	15	A
†Sgr B2	17 44 10.5	−28 21 58	60	M/A
† Sgr B2 (south)	17 44 10.5	−28 23 14	60	M/A
† W31	18 07 30.5	−19 56 29	0	M/A/E?
W33	18 11 17.9	−17 56 45	33	A
† W49N	19 07 49.5	09 01 15	14	M/A/E
†W51	19 21 23.8	14 24 52	60	M/A
K3-50	19 59 50.0	33 24 18	−20	—
†DR21	20 37 13.9	42 08 54	5	A
DR21(OH)	20 37 14.8	42 12 08	5	A
S140	22 17 41.1	63 03 40	−6	A
†NGC7538	23 11 36.5	61 11 47	−58	M
Cas A	23 21 10.0	58 32 24	0	—

* Centre of observed velocity range, LSR (local standard of rest).

† Additional measurements at offsets (in arc s) of (Δα, Δδ) = (−75, 0), (75, 0), (0, −75), (0, 75) relative to the reference position.

‡ Offsets of (−150, 0), (150, 0), (0, −150), (0, 150).

§ Observations impossible due to satellite interference.

‖ Observations marred by satellite interference.

¶ For the NGC6334 positions the nomenclature of Rodriguez *et al.*[8] was used.

Second, one finds CH_3OH maser sources (class B) close to compact H II regions of which the prototype is W3(OH). This source (as well as NGC7538) shows absorption (and thermal emission) in the J_2-J_1E lines. Towards W3(OH) the 2_1-3_0E and 9_2-10_1A$^+$ lines have been found to be masing[1,2]. A Very Large Array (VLA) study of the latter transition has shown that the maser emission emerges from unresolved spots (in a 2 arc s beam) coincident with the compact continuum emission and in the same area as the OH masers[7]. The 2_1-3_0E and 2_0-3_{-1}E emission components cover exactly the same velocity interval as the 9_2-10_1A$^+$ line (see Fig. 1) and thus it is reasonable to assume that all three masers form in the same region. Taking the upper limit on the source size obtained from the VLA observations of the 9_2-10_1A$^+$ line (1 arc s), we calculate brightness temperatures of 5-6 × 10^6 K for the stronger components of the 2_0-3_{-1} E maser.

Emission from NGC7538 in the 2_1-3_0 E and 9_2-10_1A$^+$ lines is roughly an order of magnitude weaker than in the case of W3(OH) which makes interferometer observations difficult.

Table 2 Properties of the 'strong' 2_0-3_{-1}E methanol masers

Source	Velocity range (km s^{-1})	v_{LSR}* (km s^{-1})	Δv* (km s^{-1})	∫S dv† (Jy km s^{-1})	L‡ (photons s^{-1})
W3(OH)	[−42.5, −46.1]	10 components in velocity interval		1,297	3.8 × 10^{45}
NGC6334 F	[−17, −4]	−11.22(0.01)	0.43(0.01)	1,147	2.0 × 10^{45}
		−10.46(0.01)	0.46(0.01)		
		several other components in velocity interval			
NGC7538	[−62, −55]	∼−61	∼1	198	1.5 × 10^{45}
		−56.39(0.01)	0.82(0.01)		

* LSR velocities and linewidths of strongest components are given explicitly only for cases in which fitting of a single gaussian was possible.

† Flux density integrated over the entire velocity range showing maser emission.

‡ For the calculation of the isotropic photon luminosity L, we assumed distances of 2.2 kpc (W3(OH)), 1.7 kpc (NGC6334) and 3.5 kpc (NGC7538).

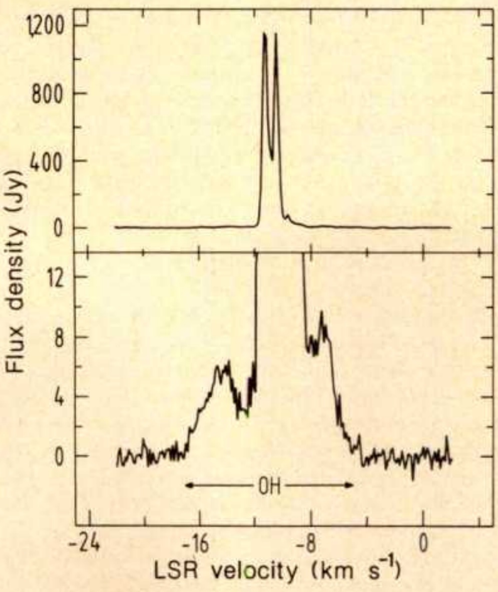

Fig. 2 The 2_0-3_{-1}E line towards NGC6334 observed with a velocity resolution of 0.12 km s^{-1}. The full velocity extent of the emission is best seen in the lower part of the figure where the intensity scale has been zoomed by a factor of 100. The velocity range showing OH maser emission[17] is also indicated.

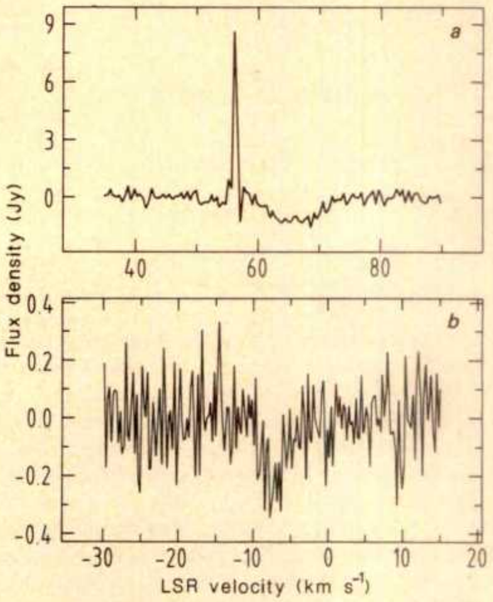

Fig. 4 Absorption plus maser emission towards W51 (*a*), and pure absorption towards S140 (*b*), showing that the 2_0-3_{-1} E line is not in all cases inverted. The velocity resolutions are 0.48 km s^{-1} in *a* and 0.24 km s^{-1} (S140) in *b*.

Thus there is no straightforward way to decide whether these lines are really masing, although this is suggested by the narrow linewidths observed. The high flux density and narrow linewidth (0.8 km s^{-1}) of the 2_0-3_{-1} E line can only be explained by maser emission and our offset measurements are consistent with a source position coincident with the compact H II region (IRS1).

Towards NGC6334 no CH_3OH lines other than the 2_0-3_{-1}E have been reported hitherto. Our offset measurements reveal, that the strongest features (at -11.2 and -10.5 km s^{-1}) emerge from positions which are, within the errors (15 arc s), coincident with the ultracompact H II region[8], NGC6334 F and the FIR source I of McBreen *et al.*[9]. They also definitely show that the position of the ~70 Jy feature at -9.6 km s^{-1} is displaced relative to the strongest features by ~1 arc min to the north, thus suggesting a relationship to the more extended continuum source E of Rodriguez *et al.*[8]. We observed absorption towards their continuum source A.

Far infrared radiation has been suggested as a pumping mechanism for OH[10] as well as for CH_3OH[2]. Menten *et al.*[11] find that the physical conditions prevailing in OH maser sources (especially W3(OH)) also favour the inversion of methanol transitions. It is therefore interesting to investigate the observational evidence for a correlation between both species.

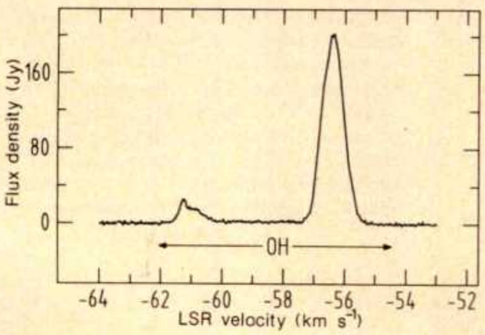

Fig. 3 The 2_0-3_{-1} line towards NGC7538. Velocity resolution is 0.03 km s^{-1}. The velocity range showing OH maser emission[13] is indicated.

A first hint of such a correlation is the overlap in the radial velocity ranges indicated in Figs 1-3. One should note that the velocity spread of the 1,665 MHz OH masers shown may be an overestimate due to the Zeeman effect[12]. Except for W3(OH), no conclusive information about spatial coincidence of CH_3OH masers with OH masers is available. Obviously, interferometer measurements are needed for the methanol lines. In NGC7538 (ref. 13) as well as in NGC6334 F (Raimond and Eliasson[14] (their source A), Rodriguez *et al.*[8]), the OH interferometer positions lie within the large single dish error boxes obtained from our 2_0-3_{-1}E observations. It is unclear from the literature whether OH emission is present towards NGC6334 E.

Finally, it should be noted that, in the case of W3(OH), the photon luminosity in the CH_3OH 2_0-3_{-1}E line is higher by a factor of ~2 than the luminosity in the OH 1,665 MHz line[10]. This suggests that the methanol abundance is comparable to that of OH and that the pump mechanism is at least as efficient. In W3(OH), the estimated OH abundance is $>10^{-5}$ (ref. 15), and Menten *et al.*[7] find a CH_3OH/H_2 ratio of $\sim 10^{-5}$. Understanding such large methanol abundances is a problem almost as challenging as understanding the maser pump.

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Evidence for a larger Sun with a slower rotation during the seventeenth century

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Historical records suggest that during the seventeenth century the Sun was subject to an anomalous scarcity of sunspots (the Maunder minimum[1]). This minimum was coincident with a severe temperature dip of the Little Ice Age[2], from which a relation between solar activity and terrestrial climate has been conjectured[3]. Solar variability is also suggested by the regular variations in the thickness of annual laminae or 'varves' in the late Precambrian formation in south Australia[4]. The climatic cycles recorded by the varves include the present 10–12-year solar cycle as well as deep minima, with a mean period of 314 years. A natural explanation of the solar cycle climate connection would be a modulation of the solar output radiation, and possibly a change of the solar diameter[5,6]. By analysing a unique 53-year record of regular observations of the solar diameter and sunspot positions during the seventeenth century, we have shown for the first time that the solar diameter was larger and rotation slower during the Maunder minimum. This finding sheds new light on the dynamo mechanism, solar variability, and the consequent effects on terrestrial climate.

J. Picard was the leader of the new school of precision astronomy[7]. He made regular observations of the Sun with a 9½-foot quadrant and 6-foot sextant, both fitted with telescopic sights[8]. He also took advantage of the invention of the filar micrometer (40,000 divisions per foot, better than 0.01 mm.[9,10]), which led to a potential accuracy of 1 arc s (1") in solar diameter determinations (the focal length of the refractor was 6 feet, and the focal solar image was about 2 cm in diameter). In contrast with Hevelius who used plain sight instruments and made qualitative drawings of the solar image, Picard obtained quantitative measurements[11] of the solar diameter, in the meridian plane (method 1), from 1666 up to his death in 1682. He also recorded the transit time of the solar image formed by a meridian refractor (denoted hereafter as method 2). The latter method was extensively used by his colleagues (Ph. and G. de La Hire) from 1683 up to 1719[12]. During each meridian transit, they measured the transit time of the prior and posterior limb of the Sun, as well as the sunspots. The transit time was recorded with an accuracy of 0.5 s, giving an error of 7" in the solar diameter. With a fixed meridian quadrant, they also measured the height of the upper limb and the sunspot, above the horizon, within ±5". It was then possible for us to reconstruct the position of individual sunspots on the solar disk, and to deduce the solar rotation. The Sun's position and orientation have been calculated from the formulae provided by the Bureau des Longitudes[13]. Subsequently, the position of a sunspot is expressed in the usual heliographic coordinate system.

Apparent horizontal solar diameters obtained from methods 1 and 2 have been calculated, and reduced to the astronomical unit, in order to correct for seasonal variations. Yearly averages of diameters are listed in Table 1 (see also Fig. 1). We distinguish two periods, one corresponding to the deep Maunder minimum (dMm), the second period related to the end of Maunder minimum (eMm), by which time sunspot activity had resumed. The first method was used at the time of dMm and later, from time to time as a check. It gives a mean diameter of 32'9" (that is 964'5" for the radius) during the dMm (see also ref. 14). Such an estimate is obtained as a mean value of 297 observing days, with a r.m.s. deviation $\sigma = 0.19''$. So, the statistical error on the mean (s.e.m.) is $\sigma/\sqrt{297} = 0.011$. The second method gives a solar diameter of 32'9" over 48 days, with a σ of 4" and a statistical error on the mean of 0.6". For the period 1683–1718, the second method was more often used: the number of observing days per year ranged from 136 to 231 days, and the standard error of the yearly average diameter never exceeds 0.47" (Table 1). During this period, the diameter shows a regular decrease down to 32'6" (that is 963" for the radius). The values of the diameter observed by La Hire at the end of the Maunder minimum are in agreement with measurements by Gascoigne[10] from 1638 to 1641, just prior to the Maunder minimum, and by Tondu[15] during the eighteenth century.

How significant are these findings? As we mentioned earlier, the accuracy of the micrometer itself should not account for more than 1" systematic error on method 1. Spherical and chromatic aberrations could also affect method 1, but this effect is unlikely to have been more than 1", bearing in mind the quality of seventeenth century optics[16]. On the other hand, the second method which relies on transit times is little affected by the geometry of the instrument. It is more sensitive to the personal bias, however, but according to Parkinson *et al.*[17] this bias does not exceed 2.2" for the horizontal diameter. The other important factor is the determination of the transit time: precision of

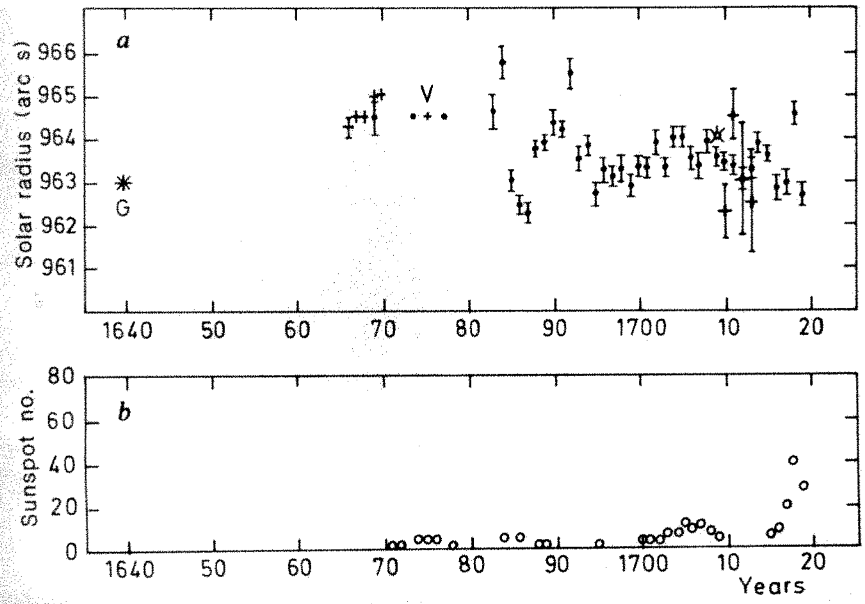

Fig. 1 *a*, Yearly averages of the solar radius during the Maunder minimum. Crosses refer to method 1 (using a filar micrometer), dots refer to the transit observations. The ✲ symbol is the diameter measured with the filar micrometer at the time of an eclipse. G symbol corresponds to observations by Gascoigne. V refers to Villiard's observations. *b*, Sunspot number along the Maunder minimum. Absence of dots means that no sunspot has been detected by the French observers.

Table 1 Horizontal solar diameter measured by Picard (method 1) and La Hire (method 2) during the seventeenth century

	Solar radius (arc s) by method 1				Solar radius (arc s) by method 2			
Year	Observing days	yearly average	s.e.m.	corrected average	Observing days	yearly average	s.e.m.	corrected average
1666	38	964.25	0.15	962.70				
1667	118	964.5	0.09	962.95				
1668	69	964.5	0.06					
1669	61	965.02	0.13	963.47	48	964.50	0.43	962.73
1670 dmM	11	965.05	0.15	963.50				
1671								
1674		964.5			110	964.50		
1676								
1683					136	964.58	0.47	962.81
1684					186	965.75	0.38	963.98
1685					162	962.98	0.24	961.21
1686					190	962.45	0.16	960.68
1687					187	962.25	0.23	960.48
1688					207	963.75	0.20	961.98
1689					189	963.88	0.22	962.11
1690					170	964.40	0.31	962.63
1691					154	964.20	0.30	962.43
1692					154	965.50	0.37	963.73
1693					178	963.55	0.29	961.78
1694					186	963.90	0.25	962.13
1695					156	962.70	0.30	960.93
1696					177	963.25	0.30	961.48
1697					181	963.13	0.26	961.37
1698					177	963.29	0.30	961.52
1699					209	962.90	0.25	961.13
1700					187	963.36	0.25	961.59
1701					226	963.28	0.24	961.51
1702					205	963.90	0.29	962.13
1703					202	963.33	0.21	961.56
1704					209	963.96	0.24	962.19
1705					227	963.93	0.25	962.16
1706					210	963.49	0.27	961.72
1707					203	963.35	0.28	961.58
1708					210	963.88	0.24	962.11
1709					202	963.55	0.21	961.78
1710	14	962.25	0.67	960.70	218	963.43	0.24	961.66
1711	25	964.5	0.60	962.95	209	963.35	0.21	961.58
1712	15	963.0	1.29	961.45	213	963.00	0.24	961.23
1713	13	962.5	1.24	960.95	202	963.40	0.23	961.63
1714					212	963.87	0.22	962.10
1715					230	963.55	0.17	961.78
1716					212	962.78	0.26	961.01
1717					229	962.95	0.24	961.18
1718					175	964.53	0.26	962.76
1719					34	962.59	0.25	960.82

Values are yearly averages and the corresponding standard error of the mean. The corrected average column gives solar radii corrected for irradiation and a second order refraction effect (in the case of transit observations). For the period 1674–76, solar diameters were derived by Villiard (an assistant of Picard), using mainly the transit timings. The results[14] are similar to those obtained by Picard during the period 1666–71.

* In 1718, an assistant observed for about 4 months, and his measurements are systematically greater than those by La Hire.

clocks was 1 s per day, so, the corresponding uncertainty would be 0.01″. In fact, the real source of error is the quantization of time by the observer, about 0.5 s on a mean transit time of 2 min, that is about 7″. This uncertainty is quite consistent with the observed r.m.s. deviation. In our reduction procedure, we have used the calculated declination of the Sun, thus eliminating the first-order refraction effect. When the observations are made at a latitude different from 90°, another correction should be applied, according to Wittman[18]. So, the yearly transit radii plotted in Fig. 1 should be decreased by about 0.25″. Both methods give a systematic increase of the solar diameter due to irradiation, which has been estimated empirically[19] to be 3.1″. Table 1 includes corrected radii. One should remember however, that this irradiation correction is somewhat uncertain and could be larger with the seventeenth century optics.

Observation of the 1715 solar eclipse by Halley has been interpreted[17,20] as leading to a diameter of about 32′0″, consistent with the modern value. An eclipse observation is intrinsically more accurate: it is less affected by the personal bias and it measures the difference between the apparent solar and lunar diameters. The departure from the corrected 1715 La Hire observations is 3.5″, greater than the 2.2″ uncertainty expected from personal bias. On the other hand, the 1715 eclipse is the only observation available, and its accuracy is strongly dependent on the precise location of the observers. Therefore, the two independent observations (La Hire data and the solar eclipse data) are not incompatible.

There remains the clear 3″ decrease of the solar diameter measured by the same skilled observer from 1683 to 1718, which is more than six times the standard deviation of the mean. The variation of the solar diameter, during the seventeenth century reaches 2×10^{-3}, that is three times larger than the uncertainty of the solar limb definition. For comparison, the change of the solar diameter over the 11-yr cycle[21] is only 2×10^{-4}.

An immediate question which arises is the following: does the variation of the Sun's diameter correspond to a real mass motion of the surface layers? The answer can be given from the study of the solar rotation.

As suggested by Maunder[7], the solar cycle continued to operate at a low activity level. During the 53 yr of observations examined by our Paris colleagues, enough sunspots were sighted so that 11-yr cycles could be reconstructed, maxima occurring in 1674, 1684, 1695, 1705 and 1716. The sunspot activity was concentrated in the southern hemisphere, except for the last cycle when the sunspots appeared in the northern hemisphere as well.

The longitude of each sunspot is calculated, assuming a rigid rotation of the Sun (a Carrington period of 27.275 days has been used). If the sunspot rotates faster (slower) than the Carrington velocity, the longitude of a given sunspot should increase (decrease) during its transit across the solar disk by an amount proportional to its excess (deficiency) velocity.

For each sunspot, we obtained the variation of longitude during its transit and we made a least mean square linear fit, the slope of which gives the mean acceleration of deceleration of the sunspot with respect to the Carrington velocity. Because of the large uncertainty in the transit time of the spot (7″), there is a large spread in the values of longitude, so we have selected only spots (~100) which could be followed for several days. In this way, we obtain a more reliable estimate of the rotational rate. Since there are few sunspots, there is no ambiguity in identifying them. A drawing of sunspots is enclosed with each observational sheet, which helps to remove any ambiguity. A few return spots have been identified: for these sunspots, the accuracy of the rotational rate is substantially increased and rule out any systematic error coming from an overestimate of the solar diameter. Synodic rotational rates of sunspots have been calculated, as well as a mean rate R for each 5° latitude belt between $-25°$ and 20°. We also plotted rotational rates by Balthasar and Wohl[22] in the period (1940–1980), and those of Hevelius calculated independently by Eddy *et al.*[23] and by Abarbanell and Wohl[24], over 1642 to 1644. The bars show estimates of the standard deviation $\sigma/\sqrt{N}$ for the mean rotation rate for each latitude belt.

Two features are evident in the rotation profile of La Hire's data (Fig. 2). During the Maunder minimum, sunspots rotate slower than their modern counterparts, by as much as 0.5° per day. Furthermore, the differential rotation is greater in La Hire's data than it is today. A very striking feature appears: the rotation profile in the Hevelius data calculated by Eddy *et al.*[23] is very similar to that of La Hire's data. The main difference is the shift velocity (0.8° per day). The difference could be real since the epoch of observations was not the same. An argument in favour of the fast velocity found on Hevelius data was suggested by dynamo theories based on the stretching of field lines by the subsurface radial gradient of angular velocity in the convection zone[25]. One class of models requires an angular velocity increasing inwards. If the high surface equatorial angular velocity arose through a redistribution of angular momentum from deeper convective layers, then the angular velocity at these deep layers should be less than for current solar cycles, thus reducing the angular velocity gradient, and possibly the strength of the dynamo action. However, observed rotational splitting of global oscillations indicates that the angular velocity does not vary much with depth[26]. Also, the discovery of a large-scale meridional circulation of convective origin[27,28] with unexpected properties questions the classical theories of the differential rotation and dynamo. A plausible alternative to the discrepancy metioned above, as suggested by Eddy *et al.*,[23] could be that Hevelius made the disk circle too small, keeping the spots on original scale. The same drawings have been observed independently by Abarbanell and Wohl[24] who found no significant difference with modern data. Cassini[29] also analysed observations of Hevelius, and some earlier data obtained by Scheiner when the Sun was still active. He noticed that the sunspots were, then, rotating with a synodic period ranging from 27 to 30 days, which conforms with the present differential rotation of sunspots. In contrast, his own observations during the 'dMm' showed a slower rotation. Once more, one should emphasize that Hevelius's drawings are qualitative, while La Hire's observations are quantitative. A larger Sun and slower rotation are quite consistent with a real pulsating phenomenon (dilation and contraction of the convective layers), over a timescale of centuries.

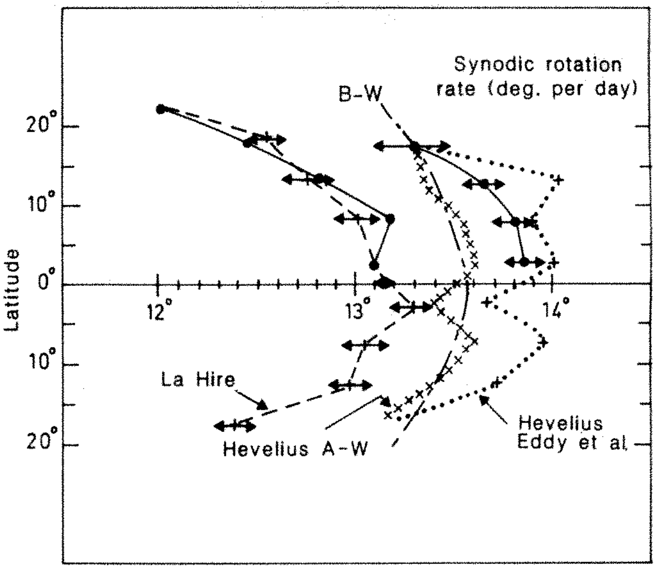

Fig. 2 Rotational rate of one hundred sunspots deduced from La Hire observations during the Maunder minimum: dashed line represents the rotational rate of sunspots in each hemisphere, while solid line is the combination of data. Dotted line and crossed line correspond to the Hevelius data (1642–1644) examined independently by Eddy *et al.*[23] and Abarbanell and Wohl[24]. For comparison, the present rotational curve is also shown (Balthasar *et al.*[22]).

The data presented here reveal that several authors have analysed historical data covering the eighteenth and nineteenth centuries and looked for secular trend in the solar diameter. Their conclusions are diverging, from no trend[17,30] to a small secular decrease of 0.2″[31,18]. None of their data extend beyond 1715 and our data cover a large fraction of the Maunder minimum, so it is not surprising to find a larger effect during this anomalous period. With the wealth of historical data collected during the seventeenth century, we have shown for the first time, that the Sun's diameter was larger than it is now. The departure from the present value (3″ at least, during the 'dMm', equivalent to 2,000 km) exceeds the possible systematic errors. On the other hand, we found that the rotational rate derived from the sunspots motion across the solar disk is significantly smaller than the present surface rotation, with an enhanced differential slippage. These two phenomena indicate that there was a real expansion of the solar envelope during the Maunder minimum, suggesting a periodic modulation of the convective zone on a timescale of several centuries[32]. Such an expansion is obviously related to the scarcity of sunspots observed during the seventeenth century. The expansion and contraction of the Sun also seem to follow an 11-yr period[21]. If they are confirmed, a new picture of the solar variability emerges: we have two distinct but strongly coupled cycles: a magnetic 11-yr (sunspot) cycle and an 11-yr convective cycle. They vary in phase; however, the amplitude of their variations is modulated over a longer period (possibly 314 yr). The fluctuations are greater on the long period than on the 11-yr timescale. We note that the solar diameter variation (2,000 km) is 20 times larger than the fluctuation of 150 km detected on the 11-yr cycle[21,31].

The connection between sunspot activity, solar luminosity and diameter has been suggested by Spiegel and Weiss[6]. If the dynamo source operates in the deep convective layers, the presence of strong fields may alter locally the adiabatic temperature gradient, modifying the convective flux transport. Resulting changes in thermal energy are large enough to produce a variation of the solar diameter and the surface effective temperature (T_e). If the energy changes were stored as gravitational potential energy, a decrease in flux through the convection zone would reduce the radius[31] and we would obtain the radius change as observed. A luminosity variation of 1% could be obtained, and this would account for the cold weather experienced in Europe during the seventeenth century[33].

Alteration of the convection will also affect the rotation of the solar envelope. We already know that surface differential rotation changes along the solar cycle (torsional oscillations observed by Howard *et al.*[34,35]. This is explained by the presence of counter-rotating azimuthal rolls[27], in the convective zone. During their movement from low to high latitudes, they accelerate or decelerate latitude belts by transport of angular momentum, causing a flexion in the rotation profile[28]. The differential rotation is greater during the minima of the 11-yr cycle, when the rolls appear to be absent. During the Maunder minimum, the differential rotation was also greater, with a low activity level. Therefore, we suggest that the large-scale convection which is responsible for the magnetic flux emergence was also absent, in relation with the Little Ice Age. Through the alteration of the rotation, the large-scale convective circulation probably plays a major role in the energy transport and the modulation of the output radiation[36].

A deceleration of 0.5% (with respect to the present value) detected on the Greenwich white-light photographic plates[22] occurred at the beginning of the twentieth century. The deceleration coincides with two cycles of low sunspot number. It would be interesting to see whether there was any corresponding change in the luminosity, through a variation of the solar diameter. If so, the convection would exhibit all the periods found in the sunspot number, including the 80-yr period, known as the Gleissberg cycle. Since the same periods are reflected in the Elatina series formed some 680 Myr ago, the modulation of the convection seems to be a permanent feature of the solar cycle. The azimuthal rolls (and related torsional oscillations) are probably the key for understanding the dynamo mechanism and the possible climatic response to solar variations.

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Ground-based infrared observations of comet Halley

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The images of comet Halley's nucleus returned during three spacecraft encounters in March 1986[1,2] are challenging the theory of the release of particles from a comet's nucleus. Instead of a spherically symmetric outflow, each spacecraft observed strong jets flowing from the sunward side of the nucleus. Here we present ground based 2–23 μm photometry and 10.3 μm imaging of Halley taken within hours of the Giotto spacecraft encounter. The photometry shows a colour temperature of 360 K and silicate emission features at 10 and 20 μm, and the image shows jet activity similar to that observed by Giotto, but on a scale of thousands of km. The expected 10 μm surface brightness, based on the particle mass distribution measured by Giotto and assuming solid, spherical grains, is a factor of six lower than the observed value. We suggest that fluffy particles could remove this discrepancy.

We observed comet Halley from 13:00 to 17:00 UT on the morning of March 13 1986 using the 2.3-m telescope of the Wyoming Infrared Observatory (WIRO). Our detector was a single-channel Wyoming Ga:Ge bolometer[3,4] with an a.c.-coupled preamplifier. All observations were made using a 7.5 Hz chopping frequency. On 13 March, Halley was 0.89 AU from the sun and 0.97 AU from earth, giving an image scale of 1 arc s = 703 km.

For the photometry (Fig. 1) we used a 5 arc s FWHM (full width at half maximum) circular diaphragm centred on the nucleus, a 20″ throw, and standard beam-switching techniques. Alpha Lyrae and Beta Pegasi were the calibrators. These data

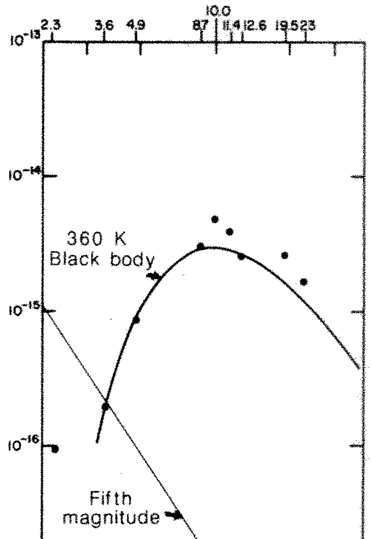

Fig. 1 Broadband 2.3–23 μm infrared energy distribution of comet Halley's nucleus on 13 March 1986. Error bars are smaller than the plotting symbols. A 360 K black-body curve has been fitted to the data. Emission features at 10 and 20 μm are due to silicates.

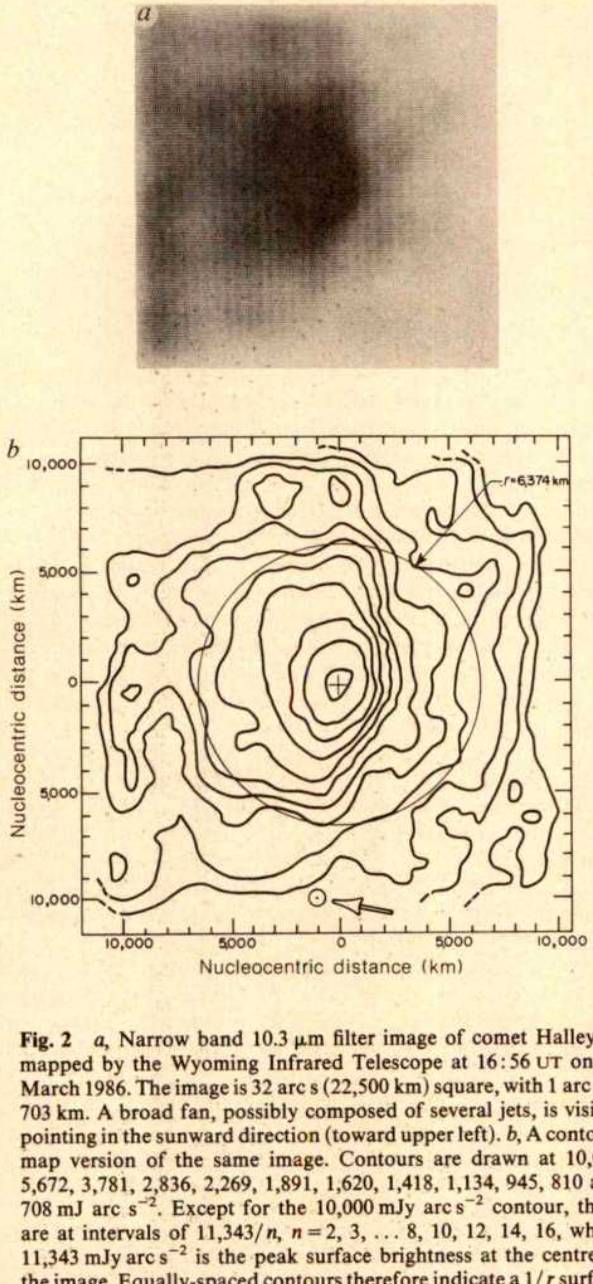

Fig. 2 *a*, Narrow band 10.3 μm filter image of comet Halley as mapped by the Wyoming Infrared Telescope at 16:56 UT on 13 March 1986. The image is 32 arc s (22,500 km) square, with 1 arc s = 703 km. A broad fan, possibly composed of several jets, is visible pointing in the sunward direction (toward upper left). *b*, A contour-map version of the same image. Contours are drawn at 10,000 5,672, 3,781, 2,836, 2,269, 1,891, 1,620, 1,418, 1,134, 945, 810 and 708 mJ arc s^{-2}. Except for the 10,000 mJy arc s^{-2} contour, these are at intervals of $11{,}343/n$, $n = 2, 3, \ldots 8, 10, 12, 14, 16$, where 11,343 mJy arc s^{-2} is the peak surface brightness at the centre of the image. Equally-spaced contours therefore indicate a $1/r$ surface brightness law. (Note the omission of every other contour beginning at $n = 8$).

are part of an extensive collection of Halley photometry taken at WIRO which is discussed elsewhere[5].

Two images of the comet and Beta Pegasi were made with a narrow-band 10.3 μm filter and the WIRO fast mapping technique[6]. The pixel size was 1 arc s, the diaphragm size was 5 arc s, and the throw was 40 arc s, placing the reference beam 40 arc s north of the source beam. About four minutes were required to build up one of these 32 × 32 pixel images. The tracking error was less than 1 arc s and the seeing was less than 2 arc s over this time.

A typical raw 10 μm image made with our technique shows a north–south intensity gradient due to changes in the detector's line of sight through the telescope as the secondary scans in declination. East-west gradients are not a problem because the entire telescope is driven to cover this dimension. Normally the resulting offsets and north–south gradients are easily determined using blank sky at the edges of the image[6]. With our Halley images, however, the edges are deep inside the coma, so an additional gradient due to the comet's emission in both the source and reference beams is also present.

To account for these two effects, we first subtracted the mean telescope background seen in the calibration images from the two comet images. By assuming a $1/r$ surface brightness law for the inner coma, we could then calculate from the raw surface brightness at each pixel (which is the difference between the brightness at that point and a point 40 arc s to the north), a value for the surface brightness S_0 at the nucleus.

We calculated S_0 for several pixels in the westernmost four columns of the two images, an area with a smoothly varying surface brightness because it is well away from any jet activity. The mean S_0 was 13 ± 1 Jy arc s^{-2} for pixels in the southern two-thirds of the image, but the S_0 values calculated from pixels near the north edge are twice as high. Other models which included offsets and linear slopes along with the $1/r$ variation produced worse results. The mean S_0 value for the southern pixels was used to calculate a single north–south baseline correction for each image that would set the westernmost four columns to the proper $1/r$ profile. The east-west variation in the ideal baseline to subtract from each north–south column is less than 20% of the surface brightness in the four columns we used.

We consider this simple model to be adequate to first order. The larger values of S_0 near the northern edge are probably a result of the very low surface brightness of the comet in the reference beam and errors in the correction for telescope emission. However, it could be due to non-$1/r$ surface brightness variations in the northern half of the image. Our data do not permit us to make more definitive statements.

After adjusting the background, the two images were registered and co-added. The resultant image was sharpened with the maximum entropy statistical method used at WIRO[7,8]. We present the final image in Fig. 2*a* and *b*. The peak central surface brightness of this image is in good agreement with the value of S_0 given above, a further indication that our simple model is reasonable.

The individual raw 10 μm images already show a broad fan extending from the sunward side of the nucleus. The processed image shown here indicates that this fan is composed of several jets, the brightest of which points 35° east of north and is visible out to about 3,000 km. The angle between lines of sight (44° about an axis through the ecliptic poles) and the rotation of the nucleus in the seven hours between the two measurements (48° assuming a period of 52.5 hours[9]) must be taken into account when comparing the WIRO image to those of Giotto. Based on the spin-axis orientation deduced from observations[9] in 1910, the dust jets seen by Giotto would have been well within view from earth at the time of our observations.

Our photometric and imaging data, when combined with the *in situ* measurements of Giotto, allow us to estimate some properties of the dust grains in Halley's coma. The observed colour temperature of the continuum emission is 360 K (Fig. 1), a factor of 1.22 over the black-body temperature at a solar distance of 0.89 AU. Assuming that the mean absorption efficiency for the coma dust grains $Q_a \approx 1$ across the solar spectrum, the superheat requires a grain emission efficiency of $Q_e = 0.45$ for the grains producing the continuum emission[10]. If the absorbing grains are graphite, those a few micrometres in size are the dominant radiators in the infrared[11].

We now estimate the expected surface brightness at 10 μm from the particle mass distribution observed by Giotto. The three Halley flyby spacecraft found several classes of dust particles, including particles rich in light elements as well as those with a CI carbonaceous chondrite composition[12,13], but for simplicity we assume most of the mass is in the form of silicate grains. The volume luminosity of such grains is

$$L_\nu = \int_0^M N(m) m E_m \, dm$$

where m is the grain mass, M is the maximum grain mass, $N(m)$ is the number of grains per unit volume per unit mass, and E_m is the luminosity per unit mass. We use

$$N(m) = 2.40 \times 10^{-6}\, m^{-5/3}\, \mathrm{g}^{-1}\, \mathrm{cm}^{-3}.$$

where m is in grams, at a radius of 6,374 km. This value was derived from a revised calibration of Giotto dust impact detection system measurements taken 7,544 km from the nucleus[14]. We scaled the 7,544 km law to 6,374 km by assuming a $1/r^2$ particle number density law in the coma. This $N(m)$ is about a factor of three higher than that based on an earlier calibration[15]. The slope of the curve is accurate only for grains in the range $10^{-9} - 10^{-6}$ g, but the effect of smaller grains is insignificant in this calculation, and we will assume that this $N(m)$ does describe larger grains which were poorly sampled by the spacecraft. The emission per mass, assuming the grains to be solid spheres, is

$$\begin{aligned} E_m &= 4\pi a^2\, \pi B_\nu(T) Q(a, T) m^{-1} \\ &= 3\pi B_\nu(T) Q(a, T)(\rho a)^{-1} \end{aligned}$$

For silicate grains we take $\rho = 2\ \mathrm{g\,cm}^{-3}$, and approximate $Q(a, T)$ by[11]:

$$Q(a, T) = 0.56a(1 - \exp(-1.2/a))$$

where the grain radius a is in microns. Although this absorption coefficient is a Planck mean over all frequencies, it is a good approximation to an integration over our 10.3 μm bandpass because the silicate cross section and the 360 K black body both peak at about this wavelength[16].

For the upper mass limit we use $M = 3.3 \times 10^{-3}$ g, a value derived from a theoretical model[15]. The resulting volume luminosity is $L_\nu = 2.2 \times 10^{-22}\ \mathrm{erg\,cm^{-3}\,s^{-1}\,Hz^{-1}}$. Integrating along a line of sight passing 6,374 km from the nucleus with an assumed $1/r^2$ law in the coma gives a predicted surface brightness of ~80 mJy arc s^{-2}.

From our photometry, the contrast of the silicate feature above the continuum is about 0.5 mag., or a factor of 1.7. We observe a mean surface brightness of ~1,300 mJy arc s^{-2} at a distance of 6,374 km, out of which ~500 mJy arc s^{-2} may be attributed to optically thin silicate emission. This is six times the predicted value.

An intriguing explanation for the large discrepancy may be that the grains are not solid spheres, but have a fluffy structure, such as seen in interplanetary dust particles[17] and suggested for cometary grains by Greenberg[18]. Solid silicate grains also could be part of an icy clathrate mixture which releases small grains when heated. Both types of particles would have higher surface area to mass ratios which would raise the predicted luminosity. The clathrate model would also help to explain the disappearance of the silicate feature in several comets at heliocentric distances greater than 1 AU (E. P. Ney and R. D. Gehrz, in preparation).

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Free precession of the comet Halley nucleus

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Images[1–3] of the comet Halley nucleus by the Vega and Giotto spacecraft and a compilation[4] of recurring events show a 'rotation' period of roughly 2.2 days. In contrast, molecular emission intensities[5] (C_2 and CN) in the inner coma exhibit 7.4-day periodic variations. In this report the comet Halley nucleus is modelled dynamically as a rigid homogeneous ellipsoid with three unequal principal axes in torque-free rotation about its centre of mass. Two qualitatively-distinct, free-precessional modes are found, each exhibiting both 2.2-day and 7.4-day periodicities. As viewed from the nucleus, the two modes are distinguished by the closed path (called a polhode) that the end of the angular velocity vector traces with a period of 7.4 days (see Fig. 1). The long-axis mode has a polhode that encloses only the long principal axis of the nucleus. It reduces to a motion proposed by Sekanina[6,7] when the minor axes are equal. However, because the polhode encloses the long axis in a long-axis mode, the resulting motion with a 7.4-day period around that axis conflicts[8] with the Vega and Giotto images of the nucleus. The short-axis mode occurs when the minor axes are unequal and the polhode encloses only the short principal axis of the nucleus. The resulting motion is around the short axis and does not conflict with the spacecraft images.

A potato-shaped[1] rigid body with unequal moments of inertia is known to freely precess[9,10] if it is spun about an axis that is oblique to its principal axes of inertia (see Fig. 1). We model the comet Halley nucleus by a dynamically-equivalent, torque-free, rigid, homogeneous ellipsoid with principal axes of unequal

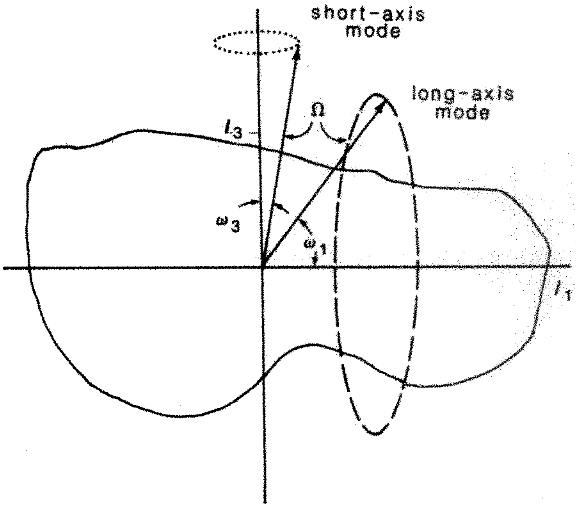

Fig. 1 The profile[8] of the comet Halley nucleus is shown together with the polhodes for the long-axis mode (dashed) and the short-axis mode (dotted).

lengths $l_1 > l_2 > l_3$. We shall follow the notation in ref. 9, and denote by Ω_i the component of the angular velocity $\mathbf{\Omega}$ along the moving ith principal axis of the ellipsoid. If $\mathbf{\Omega}$ is oblique to these principal axes, then the terminus of the vector $\mathbf{\Omega}$ traces a polhode repeating its orientation relative to the nucleus with a period T, which we shall now compute. The Euler equations[9] determining $\mathbf{\Omega}$ are

$$\dot{\Omega}_1 = -\alpha\Omega_2\Omega_3$$
$$\dot{\Omega}_2 = \beta\Omega_1\Omega_3$$
$$\dot{\Omega}_3 = -\gamma\Omega_1\Omega_2$$

where the dot represents a time derivative and the ratios,

$$\alpha = (l_2^2 - l_3^2)/(l_2^2 + l_3^2)$$
$$\beta = (l_1^2 - l_3^2)/(l_1^2 + l_3^2)$$
$$\gamma = (l_1^2 - l_2^2)/(l_1^2 + l_2^2)$$

involve moments of inertia. The solution for Ω_2 can be determined by using the two unnamed constants of the motion $\gamma\Omega_2^2 + \beta\Omega_3^2$ and $\alpha\Omega_2^2 + \beta\Omega_1^2$ to eliminate Ω_1 and Ω_3 from the second Euler equation. The long-axis and short-axis modes are distinguished by the constant (of the motion),

$$m = \frac{\alpha(\gamma\Omega_2^2 + \beta\Omega_3^2)}{\gamma(\alpha\Omega_2^2 + \beta\Omega_1^2)}$$

being less than or greater than unity, respectively. The periods of these modes are, respectively (see equation (37.12) in ref. 9),

$$T = \frac{4K(m)}{\sqrt{(\beta\gamma)}\sqrt{[\Omega_1^2 + (\alpha/\beta)\Omega_2^2]}}$$

and

$$T = \frac{4K(m^{-1})}{\sqrt{(\alpha\beta)}\sqrt{[\Omega_3^2 + (\gamma/\beta)\Omega_2^2]}}$$

where

$$K(m) = \int_0^{\pi/2} (1 - m\sin^2 u)^{-1/2}\,\mathrm{d}u$$

is a complete elliptic integral of the first kind.

As the end of the vector $\mathbf{\Omega}$ traces a polhode enclosing either the long or the short axis, we may select a convenient time such that Ω_2 vanishes and denote $\omega_i = \cos^{-1}(\Omega_i/|\mathbf{\Omega}|)$ evaluated at that time. Thus, we arrive at the formulae for the respective periods,

$$T = \frac{1}{\sqrt{(\beta\gamma)}}\frac{2}{\pi}K\left(\frac{\alpha}{\gamma}\tan^2\omega_1\right)\sec(\omega_1)\frac{2\pi}{|\mathbf{\Omega}|}$$

and

$$T = \frac{1}{\sqrt{(\alpha\beta)}}\frac{2}{\pi}K\left(\frac{\gamma}{\alpha}\tan^2\omega_3\right)\sec(\omega_3)\frac{2\pi}{|\mathbf{\Omega}|}$$

The two modes are also delineated by the argument of the elliptic integral K being less than unity. For a given total angular momentum, the rotational energy of the nucleus in each long-axis mode is greater than in each short-axis mode.

For equal minor axes the short-axis mode disappears and the long-axis mode reduces to a motion that has been considered by Sekanina[6,7]. In that motion, $\mathbf{\Omega}$ has a constant magnitude and moves at a constant angle ω_1 to the long axis, encircling it with a 'spin' period

$$T = \frac{l_1^2 + l_3^2}{l_1^2 - l_3^2}\sec(\omega_1)\frac{2\pi}{|\mathbf{\Omega}|}$$

The angle θ_1 between the long axis and the spatially-fixed angular momentum $\mathbf{M}$ is also constant, with

$$\tan(\theta_1) = \frac{l_1^2 + l_3^2}{2l_3^2}\tan(\omega_1)$$

The long axis, the angular velocity $\mathbf{\Omega}$, and the angular momentum $\mathbf{M}$ remain coplanar at all times, with that plane precessing once around $\mathbf{M}$ with a period

$$T_p = \frac{l_1^2 - l_3^2}{2l_3^2}\cos(\theta_1)T$$

Sekanina[6,7] has identified T_p with the 2.2-day period deduced from the spacecraft images[1,2] and recurring events[4]. He also identified T with the 7.4-day period of the emission intensity variations[5] in the C_2 and CN. Moreover, we identify $2\pi/|\mathbf{\Omega}|$ with the period of 2.08 days (50 h) deduced[3] from the shapes of the jets emitted by a rotating nucleus.

Spacecraft images[8] suggest that the nucleus measures roughly $16 \times 8 \times 7.5$ km with a shape that departs markedly from an ellipsoid. Indeed, the nucleus has[8] "a one kilometre deep depression near the centre of its southern face . . .". To approximate the nucleus by a dynamically-equivalent ellipsoid, we shall put $l_1 : l_2 : l_3 \approx 16:8:7$. Thus, computing α, β, and γ, we arrive at

$$T \approx 1.57\frac{2}{\pi}K(0.221\tan^2\omega_1)\sec(\omega_1)\frac{2\pi}{|\mathbf{\Omega}|}$$

and

$$T \approx 3.33\frac{2}{\pi}K(4.52\tan^2\omega_3)\sec(\omega_3)\frac{2\pi}{|\mathbf{\Omega}|}$$

in the long-axis and short-axis modes, respectively.

For unequal minor axes, the long-axis mode is qualitatively the same as in the case of equal minor axes[6,7]. In the latter motion, the nucleus spins[6] about its long axis with a period of 7.4 days, while this axis precesses about $\mathbf{M}$ with a period of 2.2 days at a precession angle $\theta_1 \approx 77°$. However, Smith *et al.*[8] find that the predicted changes in the profile of the nucleus in each long-axis mode conflict with Vega and Giotto images. That conflict stems from a particular shape that makes[8] the spatial orientation of the nucleus easily recognizable in Vega and Giotto images of adequate resolution. Observing that the profiles obtained 4.7 days apart, can allow only an integer number of rotations about the long axis over an interval of 4.7 days, the authors reject[8] a 7.4-day rotation period about the long axis.

In contrast, the profile of the nucleus in each short-axis mode is not predicted to change markedly, as the relative motion of the angular velocity $\mathbf{\Omega}$ follows a polhode around the short axis, with that axis passing through the depression on the southern face. Thus, there is no conflict with the spacecraft images. Moreover, any 'spin' period T exceeding $3.33 \times 2\pi/|\mathbf{\Omega}|$ is possible for some ω_3, because the range of $(2/\pi)K$ is $[1, \infty)$. In particular, for $2\pi/|\mathbf{\Omega}| \approx 2.08$ days as deduced[3] from the shapes of the jets emitted by a rotating nucleus, and $T \approx 7.4$ days from the Millis data[5], we have $\omega_3 \approx 11°$.

The short-axis mode, occurring only for unequal minor axes, is an alternative explanation of the conflict between the 2.2-day and 7.4-day periods[1-5] for the comet Halley nucleus. It requires less energy, for fixed total angular momentum, than the long-axis mode being proposed by Sekanina[6,7] and is not immediately rejected by the Vega and Giotto images[8].

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Balloon-borne observations of the development and vertical structure of the Antarctic ozone hole in 1986

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A springtime deficit in Antarctic stratospheric ozone has been developing over recent years[1,2]. Here we describe the vertical distribution of ozone which was measured at McMurdo Station, Antarctica (78 °S), using balloon borne sensors, on 33 occasions during the period 25 August–6 November 1986. These observations suggest a highly structured cavity confined to the 12–20 km altitude region. In the 17–19 km altitude range, the ozone volume mixing ratio declined from about 2 p.p.m. at the end of August to about 0.5 p.p.m. by mid-October. The average decay in this region can be described as exponential with a half life of about 25 days. While total ozone, as obtained from profile integration, declined only about 35%, the integrated ozone between 14 and 18 km declined more than 70%. Vertical ozone profiles in the vortex revealed unusual structure with major features from 1 to 5 km thick which had suffered ozone depletions as great as 90%.

Satellite data indicate that McMurdo was inside some portion of the vortex throughout the measurement period and was near the temperature minimum on three occasions. Preliminary in-field comparisons with standard ECC ozonesondes launched at South Pole Station, indicated general agreement during the period in which data were compared (19 August–19 September). On several occasions, the aerosol distribution was also measured. These data are currently being analysed and only brief references to preliminary results, where they appear important for interpretation of the ozone data, can be given here.

For the ozone measurements, electrochemical cells and pumps of commercial (Science Pump Corp.) ozonesondes were used. The sonde electronics were replaced with operational amplifiers and voltage-to-frequency converters to produce a digital response. A small bead thermistor was attached to the pump to determine its temperature (required for determining flow rate). The ozone and pump temperature frequencies were read into counters, the output of which were added to the serial data stream of a commercial (Vaisala Corp.) digital radiosonde which provided pressure, external temperature and humidity. A Motorola 6805 microprocessor controlled the data collection and transmission via 403 MHz telemetry. A measurement of all parameters was made at approximately 5 s intervals which provided an ozone integration of about 15 m in the vertical direction during balloon ascent. The average, e-folding response time of the electrochemical sensors is about 20 s so that sub-structures of a few hundred metres in vertical extent could be resolved. The incoming signal was decoded, recorded on floppy disk and analysed in flight using IBM PC compatible hardware. A second, remote telemetry station (free from the surrounding hills at McMurdo which limit usable range in the prevailing easterly direction) was maintained on the annual ice.

The complete ozone payload, including a 30-m reel-down device, weighed 1.8 kg. Linear, low-density, polyethylene balloons of volume 538 m^3, were used to obtain altitudes in the 30–32 km range. A number of test flights were conducted at Laramie, Wyoming, before Antarctic deployment. These included flights of dual ozone instruments which gave excellent agreement. It is estimated that the flight-to-flight comparative errors are less than 5%. The absolute accuracy depends on altitude, being worse at high altitude due to greater uncertainty in the flow-rate of the air sample and due possibly to other reasons associated with the electrochemical technique; however, in the region of the ozone deficit (12–20 km) the error is believed to be no more than 5%.

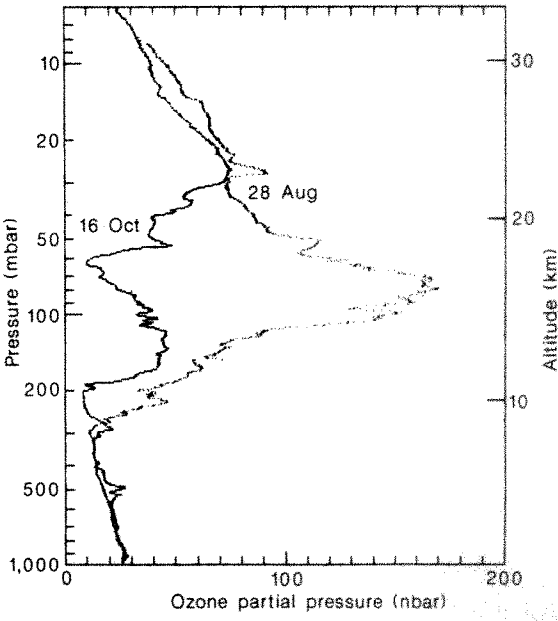

Fig. 1 Ozone partial pressure profiles (McMurdo, 1986) at the start of ozone hole formation and at the height of the depression. Temperatures in the 14–18 km region were −80°C or lower in both cases, signifying McMurdo's position as being within the region of maximum ozone depletion at these times.

Figure 1 is a comparison of ozone partial pressure profiles in the vortex before major depletion had begun and near the height of the depression. It is possible that some depletion is already present in the August profile at the 20 km level. Total ozone, obtained by integrating these profiles, was 271 and 155 matm cm including an extrapolation at constant mixing ratio above the highest point reached, of 28 and 13 matm cm, respectively. It is estimated that these values are accurate to ±5%. A Dobson spectrophotometer was not available at McMurdo for comparison. The National Oceanic and Atmospheric Administration (NOAA) Aeronomy Laboratory's visible spectrometer, operated at McMurdo during the period, mainly for NO_2 measurements but capable of total ozone measurements, indicated these to be reasonable bounds on total ozone for the period. The minimum ozone partial pressure of about 10 nbar on 16 October, at a pressure of about 60 mbar, was the lowest recorded in the stratosphere and represents a depletion in excess of 90% at this altitude. A sounding 3 hours earlier gave nearly identical results. Satellite measurements of total ozone (A. Krueger, personal communication) indicate that the ozone hole was over McMurdo on this day.

The reduction of partial pressure with altitude between 100 and 60 mbar on 16 October in Fig. 1 is faster than would occur if vertical mixing (at constant mixing ratio) were taking place. Thus, it is unlikely that upward motion resulted in this feature. In fact, none of the ozone profiles indicated any mixing of tropospheric air into the stratosphere. Aerosol measurements conducted during the period of ozone depletion showed no evidence of upward motion in the 50–100 mbar region. Quasi-horizontal advection of ozone-poor, tropospheric air from lower latitudes might account for low ozone levels below about 15 km (100 mbar pressure level) but the implication of such a process at 18 km (60 mbar pressure level) is quite doubtful. In addition, aerosol measurements on this day showed no evidence of corresponding layers as would probably be associated with such advection.

Figure 2 shows the time development of the integrated ozone in 2 km intervals. These data show clearly that the depletion was limited to the 12–20 km altitude region, and that it was most severe in the 14–18 km range. Ozone mixing ratio isopleths

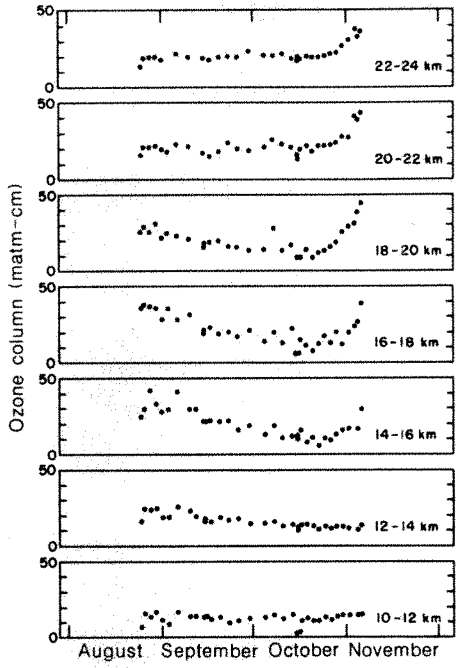

Fig. 2 Two kilometre average columnar ozone against time at McMurdo Station in 1986. Ozone decay is limited to the 12-20 km altitude region.

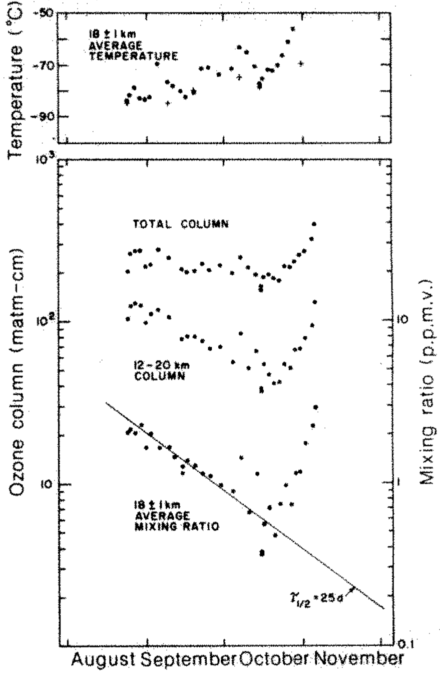

Fig. 3 Total ozone, the 12-20 km columnar ozone, and the temperature and ozone volume mixing ratio averaged between 17 and 19 km against time at McMurdo Station in 1986. The estimated half life for the mixing ratio decay during September is about 25 days. Temperatures indicated by crosses are from satellite data and signify the lowest temperatures observed at 50 mbar in the polar regions.

indicate that the depletion appeared to progress downward in altitude, beginning early and ending early in the 18-20 km region and beginning late and ending late in the 12-14 region. In the intermediate 14-18 km region, depletion continued through most of the period reaching a deficit in excess of 70% in late October. The increasing ozone trends at high altitude at the end of October are due to appearance of the high ozone ridge over McMurdo at these altitudes, probably associated with the imminent final warming.

Figure 3 gives the time development of the 18 ± 1 km average temperature and ozone mixing ratio, the total ozone column and the 12-20 km ozone column. The temperature data indicate the gradual springtime warming with excessive warming periods when McMurdo was nearer to the boundary of the vortex, notably in early September, early October and finally in late October. Satellite maps of temperature and height at 50, 30 and 10 mbar pressure, obtained within a few days of their production, confirmed the position of McMurdo relative to the temperature minimum and the centre of the low pressure system (the latter two not generally at the same location). While the decline in the total column is not impressive, amounting to only about 35%, the 12-20 km ozone column declined about 68% and the 18 ± 1 km mixing ratio about 75%. The ozonesonde data of Chubachi[3], obtained at Syowa Station (69° S) in 1982, although including only a few soundings during the critical September period, indicated a decline at 50 mbar (about 18.5 km) from about 3 to 2 p.p.m.v. or about 33%. It appears that the gradient of the decline has increased substantially in the intervening four years. Although the Syowa ozonesonde data had already indicated that most of the depletion occurred near the ozone partial pressure maximum, a sharp upper boundary of the depletion region was not apparent. The vertical ozone profiles of Hofmann *et al.*[4] in the vortex at McMurdo in early November 1985 did not include a reference profile before the depletion began. In the latter case, comparison of vortex profiles with extra-vortex profiles suggested depletion to 30 km. It is now clear that such a comparison is invalid and that one must characterize vortex profiles in August, before the depletion begins.

As indicated in Fig. 3, the mixing ratio decay during late August and September can be represented by an exponential with a half life of about 25 days (e-folding time of 36 days). It appears that motion of the ozone ridge (region of high total ozone surrounding portions of the vortex) towards McMurdo above 16 km may result in the occasional higher mixing ratios and higher 12-20 km integrated ozone values observed in October. While the available data are insufficient to draw a firm conclusion, it appears that these higher levels may also be decaying to some extent and suggest the importance of measurements in the ridge region.

As demonstrated in Fig. 4, the depletion region displayed a great deal of vertical structure especially in the 14-18 km altitude range during October. The depletion was highly heterogeneous with 2-3 km thick layers of high (>75%) ozone depletion interspersed with layers which had suffered only minimal (<25%) losses. While some of these features may be due to advection, it is unlikely that all are because they were not accompanied by correlated temperature changes or by aerosol changes when the latter were measured, which might be expected if they originated outside the vortex.

The minimum ozone region on 16 October (see Figs 1 and 4) seemed to persist for several days suggesting continental proportions although this observation may have been fortuitous since more numerous soundings are necessary to resolve the size of such a feature. It was clearly seen in soundings 3 hours apart. Wind speeds in the depleted region were about 20 $\mathrm{m\,s^{-1}}$ at this time which would suggest a horizontal extent of at least 200 km. Figure 4*a* shows an ozone layer in the heart of the depletion region which re-occurred with a 10-14 day period. Figure 4*a* also shows the depletion to be most severe at the upper altitudes (18-20 km) early on but to become a dominating feature at the lower altitudes (12-14 km) later on.

Figure 4*b* is a further example of the high degree of structure in the depletion region during October. Here four soundings, each two days apart, exhibit dramatic variations suggesting small-scale spatial structure. Although these results are from a single station, satellite data indicate that McMurdo was near the minimum temperature (and probably ozone) region in late

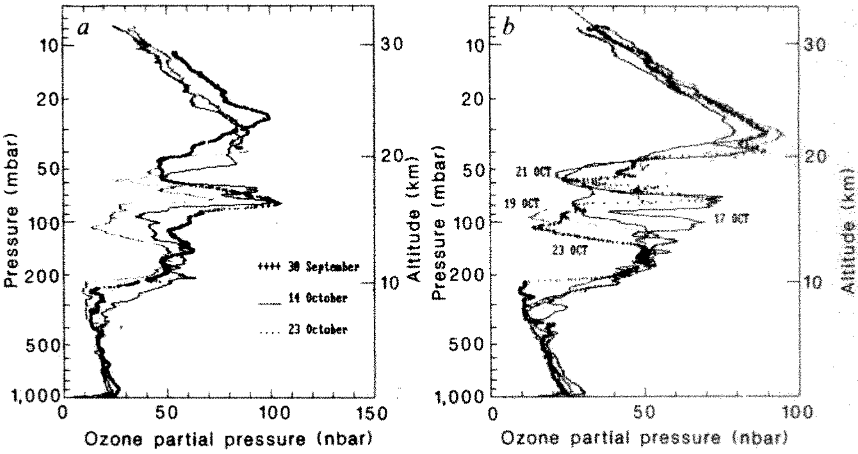

Fig. 4 *a*, Ozone partial pressure profiles which displayed a recurring ozone layer within the hole region at about 16–17 km. Temperature profiles did not show corresponding variations but were uniformly low in this region. *b*, Ozone partial pressure profiles measured 2 days apart showing the high degree of vertical structure present. Profiles such as that of 17 October indicate the presence of numerous sub-regions of depleted ozone with adjacent regions affected to a lesser extent.

August, mid-September and mid-October (see temperature data in Fig. 3). Motion of the minimum ozone region allows sampling of a large portion of the phenomenon at different times. The fact that the decay, as shown in Fig. 3, is quite uniform throughout September, suggests that on a large scale, the phenomenon is quite uniform while on a finer scale, as observed in vertical ozone profiles, considerable structure exists.

The most significant observation in this work is that the majority of the Antarctic ozone depletion occurs between the altitudes of about 12 and 20 km. The upper altitude may be as high as 22 km in the heart of the ozone hole. Subregions appear to exist in which ozone removal is highly efficient, with near total depletion. Integrated over a 2 km region at 18 km, the mixing ratio displays a half life of 25 days. A further important observation is the gradual reduction in altitude of the most efficient depletion region. These observations suggest a relationship with polar stratospheric cloud (PSC) which forms in the stratosphere when temperatures are below about −80 °C (ref. 5). The temporal variation of the ozone decrease at various altitudes in Fig. 2 almost exactly parallels the reduction of PSC extinction against time and altitude given in Hamill *et al.*[6]. It appears therefore that PSC evaporation is strongly linked to the ozone depletion phenomenon. Furthermore, the fact that some depletion continues after PSC has disappeared suggests that the evaporation process is involved.

In the cold Antarctic stratosphere, PSC is a frequently occurring optically thin haze which gives extinctions in the 10^{-3} to 10^{-2} km^{-1} range. The presence of this haze in the Antarctic stratosphere has been known for some time[7]. On the other hand, nacreous clouds are less frequently occurring distinct and smaller-scale features probably consisting of water-ice crystals. The large extinction which would be derived from optically thick nacreous clouds on relatively rare occasions are generally eliminated in satellite data analysis as the extinction would be considerably greater than 10^{-2} km^{-1} (ref. 6).

Owing to the low extinction values associated with PSC observed in satellite data, the uniformity of the extinction when temperatures are less than about −80 °C, and the estimate that a nitric acid trihydrate will condense near this temperature which is about 5 °C warmer than for pure water, Toon *et al.*[8] proposed that PSC haze is predominantly composed of nitric acid solution particles (or supercooled droplets). The latter would be smaller than particles derived from pure water due to the relative scarcity of nitric acid. The possible importance of the co-condensation of nitric acid and water was independently proposed by Crutzen and Arnold[9]. In addition to nitric acid condensation, the haze particles will accumulate nitric acid through the heterogeneous reaction of N_2O_5, formed by the reaction of NO_2 and NO_3, with water in the particle during the polar night. Through these processes, most odd nitrogen would be incorporated into PSC haze. Particle sedimentation could thus represent a sink for odd nitrogen[8].

In addition to being a sink for odd nitrogen, it is possible that inert chlorine reservoir molecules such as HCl and $ClNO_3$ may also be incorporated in PSC haze and in any nacreous (water) clouds which would form later in the winter in colder regions. Several models[10,11] have been proposed which utilize heterogeneous reactions in PSC to convert these reservoirs to active Cl_x. While the models contain no details such as the type of particle involved or the time sequence of events, and probably require that reaction products such as HOCl and Cl_2 be released from the particle before reintroduction of odd nitrogen through PSC evaporation in September, they are able to simulate the magnitude of the depletion. However, the spatial heterogeneity of the depletion, which was mainly observed in October, if not due to advection, can probably not be the result of evaporation of the uniform, satellite-observed PSC haze, nor can it result through chlorine bearing compounds escaping from the particles well before depletion begins. In the latter case, mixing would not allow the observed structure. This suggests the possible importance of more spatially limited nacreous or water cloud and again points to the critical role of particle evaporation.

If molecules such as HOCl and Cl_2 were formed heterogeneously in or on the surface of cloud particles or supercooled droplets[6] but were only released when the latter evaporated, then one can perhaps see how such large depletions in relatively small regions of space might develop since the nacreous clouds would evaporate in early spring before the nitric acid haze evaporated and active chlorine compounds released from the water clouds would have a week or two before odd nitrogen was introduced into the system by later evaporation, at higher temperature, of the nitric acid haze. However, since polar air at −80 to −85 °C may undergo warming by 5–10 °C as it circulates in the vortex during September, it is not clear that water clouds should survive very long, possibly undergoing repeated evaporation and re-condensation so that their importance is difficult to evaluate. This may not be the case for nitric acid haze which does not require as low a temperature to exist in the condensed state. Although satellite data suggest the near continuous presence of this haze in the winter Antarctic stratosphere, extinction measurements are made over pathlengths of several hundred kilometres and could mask any structure. Arctic measurements of PSC with airborne lidar have indicated the presence of fine-scale structure superimposed on broader features[6].

The measurements reported here indicate the importance of obtaining high resolution vertical profile data on both ozone and suitable tracers and have emphasized the possible significance of polar stratospheric cloud in the ozone reduction phenomenon. Modelling of the chemistry which may take place in such clouds is needed. This will require measurements of cloud particle composition and size distribution. In addition, laboratory measurements of relevant heterogeneous reaction rates will be necessary.

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Long-term climatic changes indicated by crystal growth in polar ice

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The reconstruction of palaeotemperatures from polar ice samples is essentially based on the isotopic composition of the ice. In this paper we propose a new and independent way to obtain such data by using crystal-size-change profiles. From central Antarctica Dome C and Vostok ice cores data we suggest that crystal growth rate is mainly driven by a built-in 'memory' of the surface temperature conditions at the time of deposition. A semi-empirical model of crystal grain growth is proposed, leading to Last Glacial Maximum–Holocene temperature change estimates in good agreement with isotope interpretations. However, the possible palaeoclimatic application of this model suffers some limitations connected in particular with *in situ* strain conditions.

In several ice cores, the Holocene-Last Glacial Maximum (LGM) climatic change, as depicted by isotope profiles, is characterized by a marked decrease in crystal size[1-6]. This effect has been attributed to various factors such as the *in situ* temperature history, strain conditions and the large concentration of impurities in glacial ice[1-4]. We have attempted to explain the correlation between crystal size and climate by examining the crystal-size profile along the Dome C ice core (Fig. 1*b*). The crystal growth rate (K), expressed as $\Delta(L^2-L_0^2)/\Delta t$ where L_0^2 is the extrapolated crystal size at time $t=0$ was obtained by using the chronology given by Lorius *et al.*[7]. The profile (Fig. 1*d*) shows the same features as the isotope profile (Fig. 1*c*). A growth rate of 3.32×10^{-4} mm^2 yr^{-1} was obtained from the regression line L^2 against time in the Holocene stage ($r=0.92$ for 30 values). Assuming a constant value of L_0^2 over the core, a factor of 3 in K (or 1.08 in $\ln K$) is observed between the Holocene and LGM periods.

Figure 2 shows the variation of the growth rate with the site temperature in polar firn. To the data from Gow[8,9], we have added other values for Dome C, Vostok and Dye 3. For Vostok, we used data from Barkov and Lipenkov[5] and the chronology proposed by Lorius *et al.*[10]. Results of the first 800 m of the Dye 3 core, where strain rates remain small, are from Herron and Langway[11] with the chronology given by Reeh *et al.*[12]. For Holocene firn and ice, the relationship between growth rate and temperature obtained from these data is:

$$\ln K = 20.77 - 6286/T \qquad (1)$$

where the slope value corresponds to an activation energy E equivalent to 0.54 eV, a value very close to 0.52 eV given by Gow[9].

To evaluate the possible *in situ* temperature effect on the $\ln K$ profile at Dome C, we used the method described by Duval and Lorius[6], assuming a 12 °C step at the climatic transition although the estimated variation of the surface temperature from isotope profile was only around 9 °C (refs 7, 10, 13). As shown in Fig. 1*e*, the resulting calculated growth rate is highly damped with a maximum variation in $\ln K$ of ≈ 0.30 compared with the observed variation of 1.08 (Fig. 1*d*). Moreover, the step changes observed in crystal size within depth intervals of a few decametres such as around 440 m of depth cannot obviously be explained by differences in growth temperature.

The effect of deformation on crystal growth rate is very small in the Dome C core. Indeed, the horizontal shear and vertical strain rates are lower than 10^{-5} yr^{-1} and the expected deformation mechanism is probably dislocation creep accommodated by grain boundary migration[14]. Strain energy is expected to be small compared with the driving force for grain growth. Furthermore, the typically equant texture and the absence of undulose extinction observed throughout the 905 m ice core indicates that grain growth is not modified by deformation[15].

Microparticle concentrations cannot significantly affect overall grain growth in the Dome C ice core although such an effect was observed for very thin layers in some ice cores[1,3]. The drag force induced by particles represents only a small part of the driving force for grain growth at Dome C[6]. In fact, we found that the correlation between crystal size (Fig. 1*b*) and microparticles (Fig. 1*a*) vanishes during the last deglaciation between 440 and 400 m and also below 700 m.

The Holocene-Last Glacial Maximum (LGM) transition is also marked by a concomitant change in the concentration of several soluble impurities[16-19] such as sodium, and higher concentrations being associated with colder climatic conditions. But growth rate data in Fig. 2 were obtained from inland and coastal stations for which the concentration of soluble impurities in snow can vary by an order of magnitude. For instance Na values range from about 200 to 400 p.p.b. (10^{-9} g g^{-1}) near Antarctic coast in Terre Adelie[20] to only 20 to 40 p.p.b. at Byrd[18], Dome C[17] and Vostok[19] stations. Similar concentrations (20 to 40 p.p.b.) are obtained for Dye 3[21] and Camp Century[16]. If we compare stations with similar temperature but situated differently with respect to the ocean (first in coastal area such as at Maudheim or Wilkes and second inland such as at Dye 3, Camp Century and Byrd), the respective growth rates are similar although sodium contents change by a factor of 5 up to 20. As the observed Na concentration changes along the Dome C ice core are of an order of 5 (refs 17, 22), there is therefore good evidence that sodium cannot significantly affect grain growth. With regard to other major ions, large variations in the concentration of sulphates and nitrates are seen throughout the profile of Dome C core but not especially in the LGM period[22,23].

Thus the variations in the crystal growth rate along the Dome C core do not appear to result from variations in the *in situ* temperature, strain conditions and impurity content. The strong correlation ($r=0.89$ for 120 values) we obtained between the crystal growth rate and the isotope content, including ice deposited during glacial time, and the fact that the isotope content is closely related to surface temperature[24,25] suggests that ice particles have a built-in 'memory' of the temperature at the time of deposition at the surface. From isotope data, a LGM temperature of -63 ± 1 °C was deduced[7,13]. With such a temperature, equation (1) gives a growth rate of 1.09×10^{-4} mm^2 yr^{-1}, a value which is very close to the mean observed for LGM

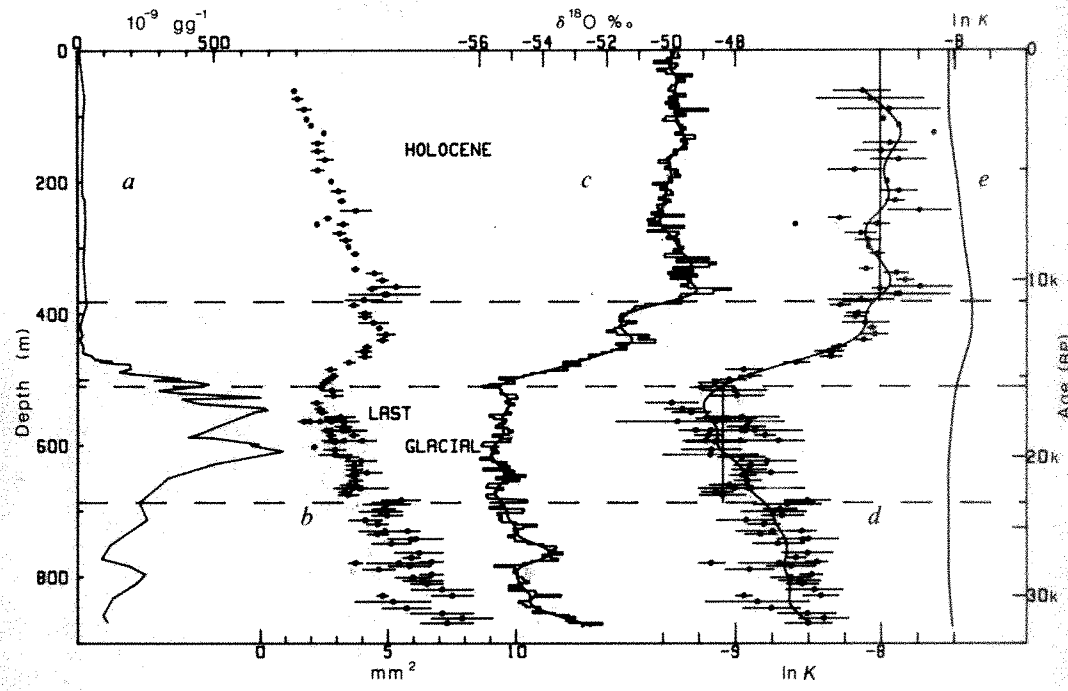

Fig. 1 Dome C ice core profiles versus depth and age. *a*, Insoluble particle content: from ref. 13; *b*, cross-sectional area of ice crystals: from ref. 6 (dots: mean values; bars: one standard deviation); *c*, isotope content (δ^{18}O% against SMOW) of ice: from ref. 7; *d*, crystal growth rate (dots: mean value; bars: one standard deviation); *e*, estimated effect of the *in situ* temperature

layers (that is $1.1 \pm 0.05 \times 10^{-4}$ mm^2 yr^{-1}). For Vostok, similarly a LGM temperature of $-65° \pm 1°$ C (J. Jouzel, personal communication) gives with equation (1), a growth rate of 0.81×10^{-4} mm^2 yr^{-1} which also compares well with the observed mean value of $0.89 \pm 0.13 \times 10^{-4}$ mm^2 yr^{-1} (ref. 5). In both Dome C and Vostok stations, the growth rate of glacial ice therefore appears to be closely related to the surface temperature during LGM. On Fig. 2, the straight line obtained from equation (1) for firn and Holocene ice, when extrapolated to a temperature of −70 °C, fits very well the measured LGM growth rates of these two stations.

A 'temperature print' can be taken by ice in the first metres of firn while densification of snow proceeds by the packing of grain simultaneously with evaporation-condensation or diffusion processes[26,27]. The high densification rate in this initial stage depends on several parameters including temperature, windspeed and the temperature gradient in the snow.

To explain the anomalous micromechanical and dielectric properties of polar ice, it was proposed that a high concentration of rotational defects (Bjerrum defects) was formed through an ageing phenomenon occurring in the first metres of firn[28]. This ageing was interpreted in terms of some sort of disintegration of ice crystals to lower their free energy. Such a process appears possible under the metamorphism of snow by surface diffusion or vapour transfer between crystals, and also because small-size crystals (< 1 mm^2) lead to grains of a high specific area. Beyond a critical density of 0.55 g cm^{-3} there is a marked reduction in the rate of densification[29]. The decrease of the specific area of grains associated with other densification mechanisms could stop the crystal disintegration. Then the structure of ice would be 'quenched'.

The grain growth is driven mainly by the curvature of grain boundaries. In such a process, the mobility of boundaries is the structure-sensitive parameter: it is directly related to the structure of boundaries via the boundary diffusion coefficient and to the crystal structure via the lattice diffusion coefficient. So, we suggest that the change in the crystal growth rate in polar ice results from variations in the crystal structure induced during the first stage of densification.

To further explain the relationship between the growth rate and temperature, we make two assumptions:

(i) The defects by which the diffusion of water molecules takes place near grain boundaries are interstitials, a mechanism recently suggested by Higashi *et al.*[30]. The concentration (c) expressed in terms of the mean site temperature T_s is: $c \approx \exp - E_f/RT_s$; where E_f is an apparent formation energy of interstitials.

(ii) The extrinsic point defects formed in polar ice near the surface can disappear only if recrystallization processes occur. In the Byrd ice core extensive recrystallization is initiated below 1,800 m of depth[1]. If, as suggested by Paterson[31], recrystallization is initiated only above a critical temperature close to −10 °C, the extrinsic interstitials would be preserved in Dome C core down to an estimated depth of about 2,600 m.

With the assumptions and with $K \approx c$, the growth rate can be written as:

$$K \approx \exp(-E_f/RT_s)\exp(-E_m/RT) \qquad (2)$$

where E_m is the activation energy for the mobility of grain boundaries and T the growth temperature. Since in the first metres of firn, T is equivalent to T_s, the apparent total activation energy E in equation (1) can be expressed as $E = E_m + E_f$. To obtain the variation of the growth rate with T_s, an estimation of the apparent activation energy E_f is needed. From observations of the growth process of dislocation loops in quenched specimens, Higashi *et al.*[30] and Goto *et al.*[32] have deduced the diffusion coefficient of self-interstitials in ice above −50 °C. They obtained an activation energy for the migration of interstitials of 0.16 ± 0.02 eV. With this value for E_m, and 0.54 eV for $E_f + E_m$, the apparent formation energy of interstitials E_f should be 0.38 eV, an estimation which compares well with 0.40 eV given by Higashi *et al.*[30]. From equation (2) it is possible to separate the influence of the surface temperature T_s from that of the growth temperature T. Taking the logarithm and then the differential of the equation and with 0.38 eV for E_f and 0.16 eV for E_m, we obtain:

$$\Delta \ln K = 0.096\,\Delta T_s + 0.040\,\Delta T \qquad (3)$$

a relationship which should be valid for cold central Antarctica.

The equation (3) based only on growth rate processes can be used as a palaeoclimatic tool. At Dome C the variation of ln K between Holocene climate and LGM is about 1.08. The calculated effect of the *in situ* temperature is 0.10 (ΔT is about 2.5 °C); so the corresponding value for ΔT_s is close to 10 °C.

For Vostok, the variation in ln K is 1.17 giving an approximately 11 °C variation in the mean surface temperature. These

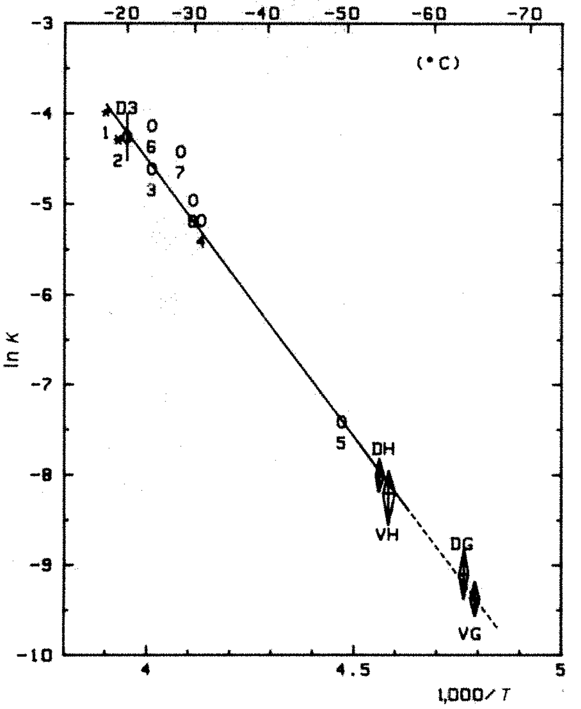

Fig. 2 Ice crystal growth rate against surface temperature in polar ice. Data are from ref. 9: Maudheim (1) and Wilkes (2) are coastal sites (asterisk); all other are from inland areas: site 2 Greenland (3), South ice (4), South Pole (5), Camp Century (6), Byrd (7), Inge Lehman (8). D3 is from the Dye 3 core (mean and one standard deviation); DH and VH are for Dome C and Vostok respectively. These last three values were obtained from ice formed under Holocene climatic conditions. DG and VG are values for Dome C and Vostok ice respectively, from Last Glacial Maximum Conditions, with temperatures obtained from isotope results. The area in each rhombus represents one standard deviation of the mean value.

results are in good agreement with the value of ≈9 °C for Dome C and Vostok deduced from an independent approach based on $\delta^{18}O$ content[7,10,13].

The values used for E_m, L_0^2 and ΔT are the main parameters included in the evaluation of ΔT_s in equation (3). For E_m a departure of ±0.02 eV from 0.16 eV gives a ±0.3 °C change in ΔT_s. LGM L_0^2 values equal to 50% of the Holocene value and LGM layer ages increased by 15% would change ΔT_s by −1.7 °C and +1 °C respectively. A ΔT value 1 °C warmer than the chosen figure would decrease ΔT_s by 0.5 °C. In conclusion, the possible variations in these parameters do not significantly change the 10 °C value we obtained for ΔT_s at Dome C and Vostok.

As regarding other long-term polar ice records, in the Byrd, Dye 3 and Camp Century profiles, grain growth stops respectively at 450 m (ref. 1), 800 m (ref. 2) and ≈300 m (ref. 2) respectively. For these cores, strain energy is high enough to prevent grain growth. Extensive undulose extinction observed in the Byrd core[1] supports this assertion. The sharp decrease in crystal size in LGM ice is accompanied by the formation of single pole fabrics in these three cores. This is an indication of ice undergoing rapid shearing. Under such conditions, the surface temperature memory effect on crystal growth is likely to be hidden.

A good check on the empirical model we propose here for the Dome C ice core and validated for the upper part of the Vostock profile will be possible when crystal size data will be available along the 2,083 m profile of this core. Of particular interest will be the crystal growth rates during the last interglacial when the surface temperatures suggested by the isotope data were even higher than the present and those during the penultimate glacial period when temperatures were similar to LGM values[10].

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The climate attractor over short timescales

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Recent work has highlighted the possibilities of using certain ideas from the theory of dynamical systems for the study of global climate. These ideas include the geometrical notion of correlation or scaling dimension, first used to analyse attractors arising in mathematical and laboratory systems[1–4] and later applied in a geophysical context[5,6]. The conclusion of the original geophysical work[5] has been criticized in the light of a reanalysis[7] which questions the existence of a low-dimensional climate attractor. Here results of a similar analysis conducted on daily meteorological observations from 1946 to 1982, are announced. This new work overcomes limitations of previous analyses, and supports the existence of such an attractor.

There has been much interest in understanding the properties of chaotic dynamical systems[1,2,4,8]. It has been suggested that strange attractors can be characterized by means of a finite time series sampled from the dynamical system. The correlation function is defined by

$$C(r) = (1/N^2) \sum_{i=1}^{N} \sum_{\substack{j=1 \\ i \neq j}}^{N} \Theta(r - |X_i - X_j|) \tag{1}$$

where $\Theta(u) = 1$ for $u > 0$, $\Theta(u) = 0$ for $u \leq 0$, and $\{X_i\}$ is the set of N sample points on the attractor constructed from a time

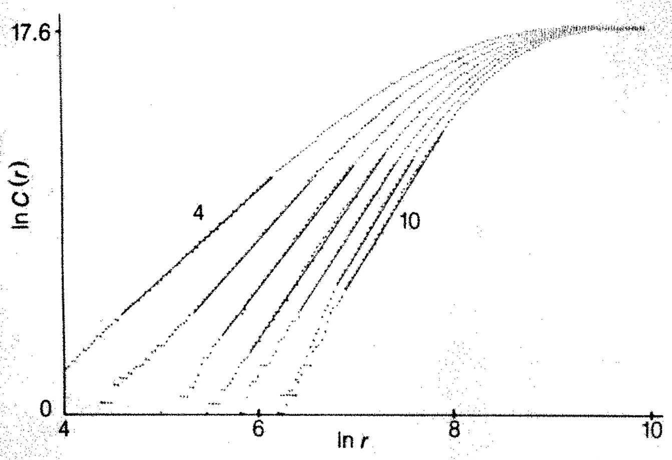


Fig. 1 Plot of ln $C(r)$ against ln r for embedding dimension, D, from 4 to 10 as obtained by method 1. Note the convergence of slopes as D increases.

series of $M \geq N$ observations. This correlation function counts the number of pairs with distance $|X_i - X_j|$ smaller than r. If for sufficiently small r,

$$C(r) \sim r^{\nu} \tag{2}$$

then ν is referred to as the correlation dimension of the attractor. If the time series consists only of the sampling of a single dynamical variable from a multivariable dynamical system, the correlation dimension still may be extracted from the series using embeddings consecutively in a sequence of higher dimensional spaces[2,3,4,9].

This number relates directly to the geometry of the attractor, and has found application in theory and experiment. Moreover, it has been used extensively to characterize the fractal properties of actual physical structures, such as aggregates of particles arising in percolation phenomena[10].

Experiments on weakly turbulent Couette flow[3] indicate values of ν for those systems between 2 and 5 depending on the Reynolds number. Measurements for some turbulent systems reach values as high as 11 (ref. 2). Thus, although a fluid could potentially have a very large number of degrees of freedom, the above studies imply that there are only a few significant degrees of freedom in such complex systems[11]. Despite the fact that it might be difficult to identify these variables, it remains of interest to determine if a system as complex at the atmosphere may be represented by a low dimensional attractor. This dimension number could function as a guide in the development of succinct theories of climate.

Nicolis and Nicolis[5] were the first to address the question of whether a climate attractor exists. They used 500 single variable values of the isotope record of deep-sea cores which cover the span of the past million years. These were interpolated from 184 actual measurements[7]. Grassberger has argued that their low value of $\nu = 3.1$ is at least in part the result of a smoothing process inherent in completing the data set and does not characterize the actual dynamical behaviour of the climate system[7]. In this work no such filtering is performed. Sets of daily local geopotential values at 500 mb taken at 12 UT extending over a span of 40 years are analysed. The data are over a timescale much smaller than the resolution of the original analysis.

Two variants of the embedding method mentioned above[1,2,4] were executed on nine sets of 12,084 measurements, 108,756 values in all. It was found that for statistical reasons, explained below, a single set corresponding to a single location was insufficient. This is to be contrasted with the 500 values used by Nicolis and Nicolis[5] or the number of values (that is, $M \leq$ 7,100) used in the other analyses of this kind[6,7].

For each embedding dimension, D, we obtain C as a function of r. The region in r for which

$$C(r) \sim r^{p} \tag{3}$$

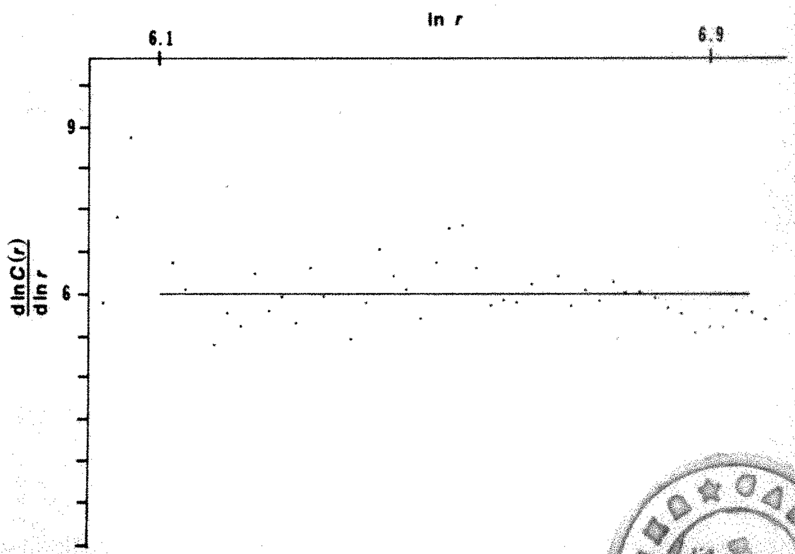
is isolated. This will not cover all r, as a data set has a finite diameter. As well at the other extreme, the finite resolution and sparseness of the measurements limit the smallest value of r for which the correlation function can have this behaviour. If p approaches a limiting value ν as D is increased, ν is identified as the correlation dimension.

Care must be taken in carrying out the above programme. Too small a magnitude of M, may invalidate the procedure. We wish to emphasize that the very existence of this scaling region depends on an adequate value for M.

In order to understand this, one must begin by recognizing that a particular data set need not exhibit scaling in the form of the asymptotic equation (3) for all or any values of D. In fact, the only basis for assessing the actual compliance with equation (3) is the quality of a straight line fit through a finite set of points. With an apparently plausible, but erroneous fit of a straight line to a plot of ln $C(r)$ versus ln r, it is thus possible to obtain spurious values of p. If a line is fitted to intervals of r for which r is too large, the resulting value of p inferred from such an erroneous fit will be too small, because for large values of r, approaching the diameter of the finite set $\{X_i\}$, $C(r)$ saturates, tending towards a constant value. In practice, this situation can be diagnosed by the inability to get a good straight line fit.

Since the number of points within a radius r of a given point

Fig. 2 A sample plot of d ln $C(r)$/d ln r against ln r showing the scaling region for embedding dimension 9 as determined by method 2.



varies within limits as a positive power of r, the population of pairs on smaller scales will be much less than those on larger scales. If N, the number of points, becomes smaller for a fixed D the population of pairs over the scales for which equation (3) holds will begin to be depleted. Eventually, total depletion on small scales, and large fluctuations in $C(r)$ because of low populations at slightly larger scales will mask the scaling region entirely. Unfortunately, the remaining segment of $C(r)$ at still larger values of r will be somewhat saturated. Any fit to such a curve in this region will result in a value of p less than its true value, as explained above.

For a fixed M the number of pairs varies with embedding dimension as D^{-2}, while the overall 'capacity' of the embedding space increases with D. Therefore with increasing embedding dimension the relative sparseness of the points will be accentuated on both accounts. The interval in r for which equation (3) holds is consequently squeezed between the intervals of depopulation and saturation until at some critical value of D, D_c, equation (3) ceases to hold for any r. Any apparent fit of a straight line to such a curve for $D > D_c$ will probably result in an underestimate of p. Thus one might obtain a sequence of underestimates that can lead to a false convergence of p. For the 500 mb geopotential values, with $M = 12{,}084$, it was found that $D_c \approx 5$. That is, the daily values over a forty year interval from one location were insufficient to complete the analysis. In other words, only the values of D from $D = 1$ to $D = 4$ exhibit points on a correct graph of p against D. No points for $D > 4$ exist in this instance, since no good scaling region was evident.

In our analysis we took measures to increase our value of D_c beyond the values of D that we analysed. We began by making a boundary correction. $C(r)$ from equation (1) is referred to as a correlation function; however, in this context this name is perhaps misleading: we only perform the outer sum in equation (1) in order to improve the statistics. In principle, any one of the inner sums would reveal the behaviour of $C(r)$ as in equation (3), if there were enough data.

The intervals over r for which scaling occurs will vary strongly from one inner sum to another. An expansion point which is close to the boundary of the set can be surrounded by others only for very small values of r. Such points contribute to the saturation in $C(r)$ when r is still very small. These offending points may be judiciously discarded in the outer sum to produce a boundary corrected function $C(r)$. This has the effect of increasing the range in r over which equation (3) holds, and effectively increases D_c. For our data D_c appeared to increase by 1 or 2. This was not enough of an increase in D_c for our case. With too strong a correction the statistics on low scales suffer badly even if larger r values participate in the scaling. The result then is simply to displace the scaling interval to larger scales.

In addition to the boundary correction we took the more important step of increasing the overall effective value of M. We incorporated into the calculation the daily data from eight locations surrounding the original data site. All nine sites fall within a geographic region located in eastern North America, and cover only approximately one per cent of the Earth's surface. We incorporated the extra data into the calculation in two distinct ways. In the first method (method 1), points X_i, in the D-dimensional embedding space were created by choosing D consecutive (daily) measurements as coordinates. The data from each location were treated together as independent measurements from a single site by concatenating the different time series from each site to create one large time series. The results of this method are indicated in Fig. 1 and summarized by the crosses in Fig. 3. Figure 1 shows that a straight line fit to the $\ln C(r)$ against $\ln r$ graphs is good over a reasonable range of r.

The other method (method 2) entailed the use of the data from each site as a separate coordinate of a point. The embedding was done by introducing the data from progressively more sites as additional coordinates. In this way the number of points N for each D remained constant up to $D = 9$. The results are indicated in Fig. 3 by the triangles. Figure 2 shows the value of p at each r for $D = 9$ using this method. It is clear that the scaling region, appearing as a plateau marked by the horizontal line in this diagram, is still strongly represented at $D = 9$, from which we conclude that D_c has not been exceeded.

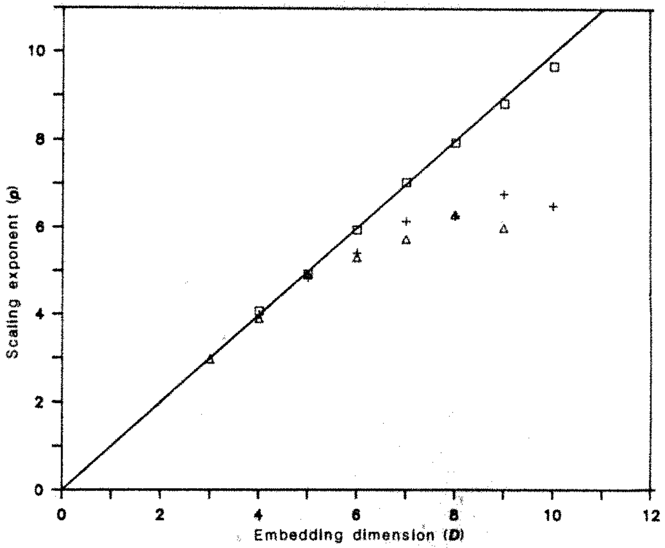


Fig. 3 Summary of results of scaling exponent, p, as a function of embedding dimension, D, obtained by two methods (method 1, +; method 2, △). A random number set of the same size as the data set was used as a control (□). Note the saturation of the exponent arising from the data, for both methods, while there is no saturation for the random number set. This limiting value is identified as ν.

Figure 3 demonstrates that despite the rather different methods employed in selecting points, both methods yield similar results, that is, a similar convergence in p leading to a value of ν slightly in excess of six. The value of ν suggests that on short timescales (that is, decades) climate might be represented as a system with as little as seven degrees of freedom. Thus we agree with the original position of Nicolis and Nicolis[5] that the atmosphere and oceans exhibit properties of a low dimensional attractor, although our analysis deals with timescales much shorter than theirs.

Fraedrich[6] has apparently also obtained evidence for a low dimensional climate attractor. However, no convergence of p was obtained until, perhaps unjustifiably, winter and summer pressure readings were separated. Further, according to our reckoning, the number of measurements used might be considered at best barely adequate for his result.

Since our results are based on measurements fairly localized on the Earth, the question arises as to whether the value of $\nu \sim 6$ for this attractor is only locally valid. Work on the global nature of the climate attractor is in progress.

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Effects from equation of state and rheology in dissipative heating in compressible mantle convection

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The thermal profile in the Earth's interior is influenced by many factors. One of the least understood and studied processes is that resulting from adiabatic heating and viscous dissipation. With the exception of the work by Jarvis and McKenzie[1] very little has been done on the effects of compressibility on mantle circulation. It is quite common for geophysicists to add the adiabatic temperature gradient *a posteriori*[2] to temperature profiles derived from Boussinesq (incompressible) equations. Recently it has been shown[3] with the mean-field method[4] that there exists a strong coupling between the rheological parameters, such as the activation volume, and the thermodynamic constants governing adiabatic heating. Here we point out the important consequences brought about by incorporating the effects of equations of state and rheology in the dissipative heating term of the energy equation. We demonstrate explicitly the ways in which compression may raise the interior mantle temperature and illustrate how this effect can, in turn, be used for constraining some of the intrinsic parameters associated with the equation of state in the mantle.

We have employed the mean-field equations for studying steady-state behaviour, which should yield important information about the basic physics of the processes involved. Although it is an approximation to the full set of convective equations, the mean-field approach has increasingly been used by geophysicists to obtain preliminary ideas about various convection problems, ranging from variable-viscosity[5] to double-diffusive convection[6].

We have derived the single-mode, mean-field equations appropriate for an elastic, compressible convection[1] with variable viscosity in the limit of infinite Prandtl number. Within the present approximation, viscosity depends on horizontally averaged temperature and pressure. A depth-dependent equation of state $\rho(z)$, which increases exponentially with depth, is used for prescribing the background density profile. We have non-dimensionalized, with respect to the whole mantle, depth d ($d = 3{,}000$ km) and have chosen a z-axis pointing downward. The relevant steady-state equations may be written as the momentum equation

$$\begin{aligned}&\frac{\mathrm{d}^4W}{\mathrm{d}z^4}+2\frac{\mathrm{d}^3W}{\mathrm{d}z^3}\left(\frac{1}{\eta}\frac{\mathrm{d}\eta}{\mathrm{d}z}-2\frac{D}{\gamma}\right)\\&+\frac{\mathrm{d}^2W}{\mathrm{d}z^2}\left(\frac{1}{\eta}\frac{\mathrm{d}^2\eta}{\mathrm{d}z^2}-5\frac{D}{\gamma}\frac{1}{\eta}\frac{\mathrm{d}\eta}{\mathrm{d}z}+5\frac{D^2}{\gamma^2}-2k^2\right)\\&+\frac{\mathrm{d}W}{\mathrm{d}z}\left(3\frac{D^2}{\gamma^2}\frac{1}{\eta}\frac{\mathrm{d}\eta}{\mathrm{d}z}-\frac{D}{\gamma}\frac{1}{\eta}\frac{\mathrm{d}^2\eta}{\mathrm{d}z^2}-2k^2\frac{1}{\eta}\frac{\mathrm{d}\eta}{\mathrm{d}z}-2\frac{D^3}{\gamma^3}+4k^2\frac{D}{\gamma}\right)\\&+k^2W\left(\frac{1}{\eta}\frac{\mathrm{d}^2\eta}{\mathrm{d}z^2}+k^2+\frac{D}{\gamma}\frac{1}{\eta}\frac{\mathrm{d}\eta}{\mathrm{d}z}-\frac{2}{3}\frac{D^2}{\gamma^2}\right)\\&+k^2\exp\left(2\frac{D}{\gamma}z\right)R\frac{\theta}{\eta}=0\end{aligned}\tag{1}$$

The temperature field is decomposed into a horizontally averaged part $\bar{T}(z)$ and a fluctuating component $\theta(z)\cos kx$, where k is the dimensionless wavenumber of the assumed roll along the x-direction. The equations for the mean and fluctuating temperatures in which the weak-coupling approximation[4] is assumed for the divergence of $\boldsymbol{u}\theta-\langle\boldsymbol{u}\theta\rangle$, take the form

$$\begin{aligned}&\frac{1}{\rho(z)}\frac{\mathrm{d}^2\bar{T}}{\mathrm{d}z^2}-\frac{\mathrm{d}}{\mathrm{d}z}(\theta W)+D\theta W+\mu\\&+\frac{D\eta}{R}\exp\left(-3\frac{D}{\gamma}z\right)\left[\left(2\frac{\mathrm{d}W}{\mathrm{d}z}+\frac{D}{\gamma}W\right)^2\right.\\&\left.+k^2\left(W+\frac{1}{k^2}\frac{D}{\gamma}\frac{\mathrm{d}W}{\mathrm{d}z}-\frac{1}{k^2}\frac{\mathrm{d}^2W}{\mathrm{d}z^2}\right)^2+\frac{1}{3}\left(\frac{D}{\gamma}W\right)^2\right]=0\end{aligned}\tag{2}$$

and

$$\frac{1}{\rho(z)}\left(\frac{\mathrm{d}^2\theta}{\mathrm{d}z^2}-k^2\theta\right)+D(\bar{T}+\bar{T}_0)W-W\frac{\mathrm{d}\bar{T}}{\mathrm{d}z}=0\tag{3}$$

In equations (1)–(3), the mean viscosity profile is given by η. The Rayleigh number R is based on the interior viscosity η_∞. The parameters D and γ are respectively the dissipation number and Grueneisen parameter associated with the equation of state[1] which is

$$\rho(z)=\rho_0\exp\left(D/\gamma\right)z),\tag{4}$$

where ρ_0 is the surface density. The scale-height for the adiabatic temperature is given by $1/D$, whereas that for the density is

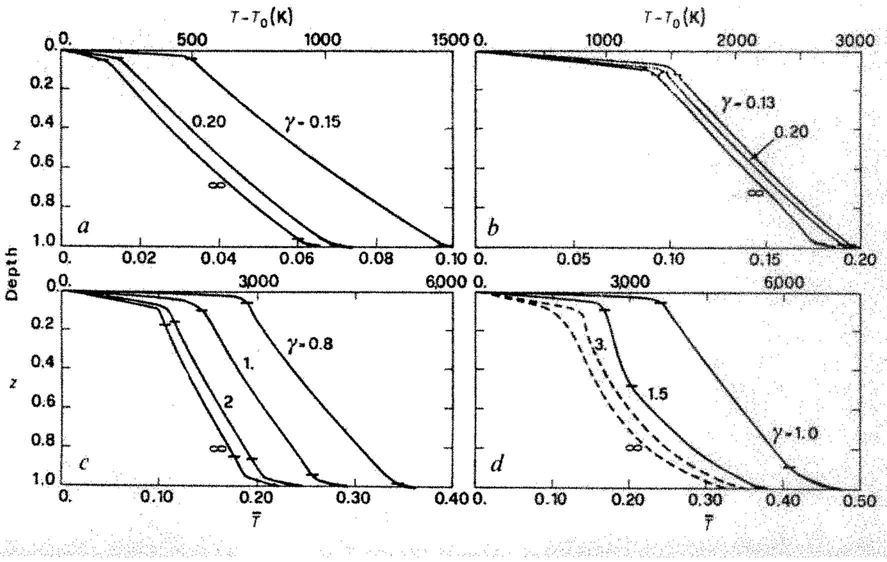

Fig. 1 Mean temperature as a function of depth for various rheologies. *a*, Constant viscosity, $\eta=\eta_\infty$; *b*, temperature-dependent viscosity, $\eta(\bar{T})$; *c* and *d*, temperature- and pressure-dependent viscosity, $\eta(\bar{T},p)$. A heat flux, based on the conduction flux from volumetric heating with strength H, is imposed at the bottom. The Rayleigh number for variable viscosity is based on the asymptotic viscosity η_∞, and is 10^9 for all cases. The temperature has been non-dimensionalized by $\bar{T}=T-T_0/T_D$ where $T_D=\rho_0Hd^2/k_0$. The density ρ_0 is set to 3,400 kg m^{-3}, $H=10^{-12}$ W kg^{-1} and $k_0=4$ W m^{-1} K^{-1}. The surface temperature T_0 is set to 1,100 K for all cases to simulate the effects of lithospheric lid from variable viscosity. $D=0.5$ and $\mu=0$. The rheological parameters correspond to $Q^*=50$ kcal mol^{-1} in *b*, *c* and *d*, $V^*=4$ cm^3 mol^{-1} in *c* and $v^*=8$ cm^3 mol^{-1} in *d*. An aspect ratio of one is asumed for the convective roll.

prescribed by γ/D. $\bar{T}_0$ is based on the surface temperature T_0, an intrinsic parameter in compressible convection. The parameter μ expresses the rate of internal heating. For a situation with a bottom temperature T_B specified and a given amount of internal heating H, $\mu = R_H/R_B$, where R_H represents the Rayleigh number describing a purely internal-heating configuration with an adiabatic bottom[7] and R_B is the Rayleigh number associated with a base-heated convection with a temperature difference of $T_B - T_0$. For a bottom thermal condition with a given flux F the partitioning ratio μ becomes $\mu = \rho_0 Hd/(\rho_0 Hd + F)$.

In the momentum equation both the horizontal gradients of temperature and perturbation pressure are included in the generation of the vorticity. The adiabatic heating term, which is proportional to D and the vertical thermal advection, appears in both the mean and fluctuating temperature equations.

We use a viscosity law, non-dimensionalized with respect to η_∞, of the following form

$$\eta(T, z) = \exp\left(\frac{H^*}{RT} - \frac{Q^*}{RT_\infty}\right) \tag{5}$$

where η_∞ is taken to be 10^{22}P, the activation energy is denoted by Q^*, the activation enthalpy by $H^* = Q^* + \rho(z)gzdV^*$, the activation volume by V^*; T is in Kelvin, T_∞ is 2,773 K and R is, in this equation only, the gas constant. The parameter T_∞ represents a characteristic temperature in the lower mantle. An activation energy of 50 kcal mol^{-1} is used for the purpose of illustrating the strong temperature dependence of the viscosity, and activation volumes between 4 and 8 cm mol^{-1}, characteristic of deep mantle material[8], have been employed. An aspect-ratio one cell ($k = \pi$) has been assumed throughout, as have been the stress-free boundary conditions at the top and the bottom. Our results are relatively constant for k between 1.5 and 9. The temperature at the surface is kept constant at $T_0 = 1{,}100$ K, thus imposing $\theta = 0$ there. At the bottom we have set $d\theta/dz = 0$ and have employed $d\bar{T}/dz = 1 - \mu$ for heating configuration with a specified heat flux and $\bar{T} = 1$ for the fixed bottom temperature situation. We have solved the nonlinear system (1) to (3) with the associated eight boundary conditions on $\bar{T}$, w, θ by decomposing them into a coupled system consisting of two fourth-order, boundary-value problems. Two hundred grid points have been distributed in an adaptive manner.

The effects of volumetric changes on dissipative heating are manifested by terms involving D/γ in equation (2). From scale analysis this contribution Φ_B from the equation of state in the shear-heating term is predicted to behave as

$$\Phi_B \sim \eta_M u_M^2 D^2/\gamma^2 \exp\left(\frac{D}{\gamma} z\right) \tag{6}$$

where η_M and u_M denote the characteristic viscosity and velocities respectively in the interior. It is clear that there will be a growing amount of shear heating, as one increases the compressibility or, conversely, decreases γ sufficiently.

Figure 1 shows the mean temperature profiles for various rheologies ranging from one with constant viscosity to a temperature- and pressure-dependent one. A constant heat flux is supplied at the bottom. A moderate value of the dissipation number is used, and the effects of compressibility are brought out by varying γ from ∞ to $\sim$0.1. The extreme cases would correspond to a change in density by a factor >10. The tick bars delineate the portions of the thermal profile which lie along the adiabatic gradient. Dashed curves (Fig. 1*d*) represent super-adiabatic states. Note that in the mean-field approximation viscous dissipation is not entirely balanced by adiabatic heating in the steady equation for the mean temperature. This approximation leads to an increased temperature gradient at the top boundary. The differences in the interior temperature between mean-field and two-dimensional results[1] increase with the amount of convective vigour for constant viscosity[3].

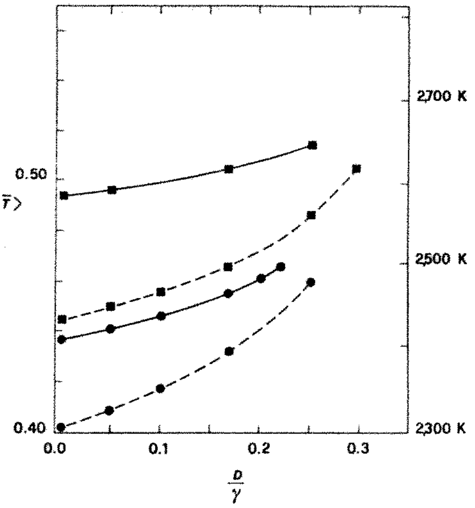

Fig. 2 Effects of varying the Grueneisen parameter on the vertically averaged temperature, $\bar{T}$. The dissipation number D is constant at 0.5. The bottom is kept at a constant temperature of 4,100 K; T_0 is the same as in Fig. 1. The temperatures on the right hand side correspond to the averaged temperature given in dimensional form. The nondimensional temperatures on the left are scaled by the temperature drop across the mantle which is here taken to be 3,000 K. The rheological parameters correspond to $Q^* = 50$ kcal mol^{-1} and $V^* = 4$ cm^3 mol^{-1}. The basal-heating Rayleigh number R_B appears in both the momentum and energy equations, whereas the internal-heating Rayleigh number R_H appears only in the mean temperature equation. They are both nondimensionalized by η_∞. An aspect ratio of one is used. - - -, $R_H = 0$; ——, $R_H = R_B$; ■, $R_B = 2 \times 10^8$; ●, $R_B = 10^9$.

The convective strengths of the runs presented in Fig. 1*a*–*c* are $\sim 10^5$ times supercritical. For both constant and temperature-dependent viscosities (Fig. 1*a*, *b*) there are no discernible effects from compressibility until γ becomes <0.2. As the Grueneisen parameters of mantle substances lie between 1 and 3 (refs 9, 10) these results suggest that shear heating in the presence of compressibility does not result in major changes to the geotherm for constant[1] and temperature-dependent rheologies.

On the other hand, the situation is quite different with the introduction of pressure dependence into the rheology. The shear heating now peaks sharply in the lower mantle. The interior of the mantle can be warmed up by several hundred degrees above the incompressible ($\gamma = \infty$) profile, for reasonable values of γ (Fig. 1*c*) corresponding to density changes of around 30% across the mantle. For activation volume $V^* = 4$ cm^3 mol^{-1} there is a rapid increase in the interior temperature as γ drops from 2 to 1. For $\gamma < 1$, the steady-state solution jumps to a higher branch in which the interior temperature is several thousand degrees hotter. In these cases there is a sharp focusing of viscous heating at the base of the mantle. Convection becomes more sluggish[6] with larger activation volume[3], leading to a hotter interior (Fig. 1*d*). However, the transition value of the Grueneisen parameter γ_c still remains within geophysically reasonable limits, between 1 and 2. We remark here that the temperature estimates are based on the cartesian formulation and that effects from sphericity[11,12] would reduce these interior temperatures by around a factor of two in the case of constant viscosity. The transitions at low Grueneisen parameters is also found for Rayleigh numbers 50 and 100 times smaller than the ones reported here.

The combined effects of both basal and partial internal heating configurations are shown in Fig. 2, where the averaged temperature is plotted as a function of D/γ for purely base-heated (dashed curve) and partially internally heated (solid curve) cases. In the latter case the internal-heating Rayleigh number R_H is set equal to the basal-heating Rayleigh number R_B ($\mu = 1$). A temperature difference of 3,000 K has been imposed across the mantle. We observe a gradual increase of the interior temperature with increasing compressibility, as D is kept constant at 0.5. The temperature rise because of shear heating is greater in the case without any radioactive heating. The last points, shown on the right, mark the regime in which heating effects from volumetric deformations are still small. We observe that, for this set of rheological parameters ($Q^* = 50\ \text{kcal mol}^{-1}$ and $V^* = 4\ \text{cm}^3\ \text{mol}^{-1}$), the averaged temperature of the mantle cannot exceed 2,700 K, before transition to a much hotter, geophysically unrealistic state would occur for D/γ, greater than about 0.3 or $\gamma_c = 1.7$. As this γ_c lies within geophysically plausible bounds, this would argue for the potential importance of employing the equation of state in conjunction with temperature- and pressure-dependent rheology in compressible convection models for constraining the thermodynamic parameters D and γ of the deep mantle. Conversely, one may also put bounds on the rheological parameters Q^* and V^* of the lower mantle, if the equation of state parameters can be estimated with greater precision.

We conclude that the coupling between variable viscosity and equation of state in dissipative heating is potentially an important mechanism in mantle convection. The fundamental concept that rheology, equation of state and radiogenic heating are all linked to each other by virtue of nonlinear thermomechanical couplings must be strongly emphasized as a consequence of these findings. To explore further the implications of this phenomenon for the Earth's thermal behaviour, we need to corroborate these results with the full equations and also with depth-dependent thermal expansion coefficient and thermal conductivity, which would have important implications for the D^{11} layer. With the next generation of supercomputers, the spatial resolution required to understand this nonlinear process in two or three dimensions is well attainable.

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Rupture and inflation of a basic magma chamber by silicic liquid

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The prevailing view among volcanic geologists is that the introduction of basic magma into a silicic magma chamber may trigger explosive silicic volcanism[1] and lead to the eruption of mixed magmas[2]. Although the patterns of basic and silicic eruptions in some areas may support an input of basic magma[1] just before silicic eruption, evidence from the study of the erupted rocks is generally equivocal; many occurrences are as readily explained by injection of silicic into basic magma. Although this latter sequence is a more efficient way to produce contemporaneous magma mixing and explosive silicic volcanism, it appears to have been largely overlooked. This report describes evidence in the Newark Island layered intrusion for the rapid emplacement of large volumes of silicic magma into a basic magma chamber and suggests that such events, in the uppermost crust, may also be important in triggering explosive silicic volcanism and commingling of magmas just before eruption.

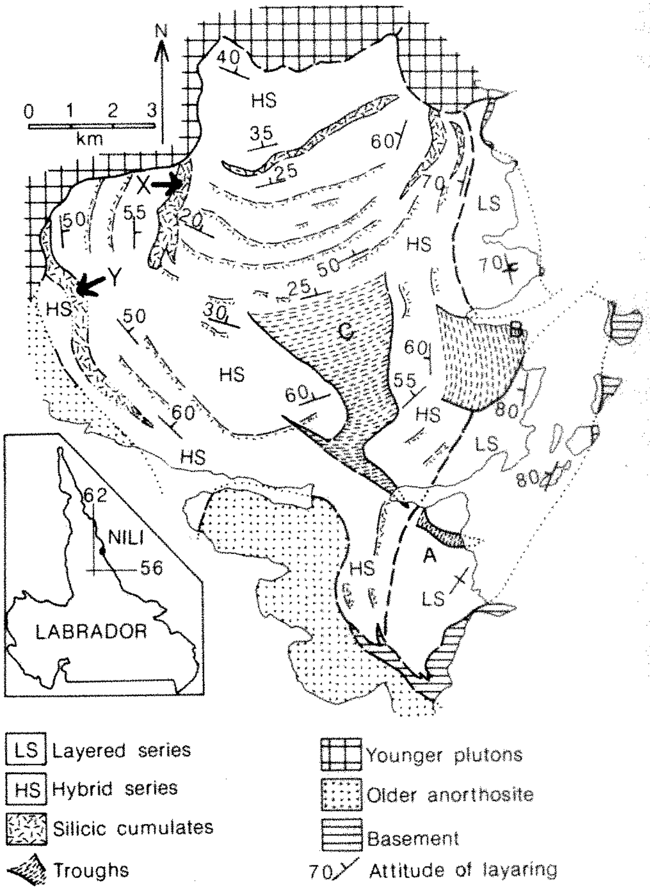

Fig. 1 Simplified geological map of the Newark Island layered intrusion. Troughs truncate and displace cumulates (both LS and HS) and consist largely of massive gabbroic rocks with walls lined by granite. They originated as silicic feeders, which were then filled by collapsing resident basic magma.

Layered intrusions, as solidified magma chambers, provide direct evidence for the sequences and compositions of replenishment and mixing events that are sometimes inferred from erupted rocks. Clear proof of injections of basic magma into a silicic chamber can be seen where chilled basic pillows and sheet-like masses are interlayered with silicic cumulates[3,4]. However, when silicic magma is emplaced into a basic magma chamber, it rises rapidly to the top of the chamber and may leave a less obvious record of its passage within the cumulates. If the temperature contrast is great, some resident basic magma will be chilled during the rise of silicic magma and sink to the chamber floor as pillows resting in mafic or hybrid cumulates. Such features are common in the hybrid layered intrusions of the Nain anorthosite complex[5,6].

The Newark Island layered intrusion (NILI) is ~150 km² in area (Fig. 1) and was emplaced at a depth of ~8–10 km (ref. 7). Its original floor, now the eastern contact, rests on Archaean metamorphic rocks; its top is truncated by younger plutons. The Layered Series (LS) consists mainly of gabbroic cumulates, with plagioclase and olivine the dominant cumulus phases[8]. Current-related structures indicate that when these rocks were deposited, the chamber floor dipped moderately to the west. Reversals in mineral composition trends indicate that the magma chamber was periodically replenished by basic magma.

The Hybrid Series (HS) consists mainly of many repetitions of gabbroic cumulates which grade upward through heterogeneous pyroxene diorite and monzonite to rocks with cumulus quartz. During deposition of the HS, the magma chamber

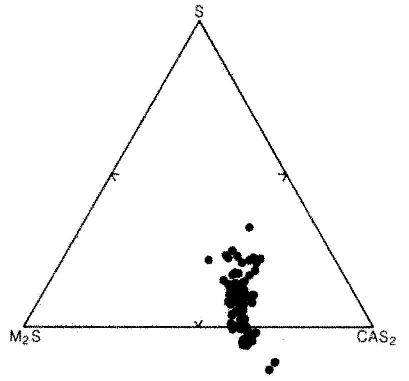

Fig. 2 Compositions (mol %) of 90 dykes and chilled pillows projected into CMAS (CaO-MgO-Al_2O_3-SiO_2) after Grove *et al.*[15]. M_2S, olivine; CAS_2, plagioclase; S, quartz; compositions are projected from CMS_2 (clinopyroxene). Compositions lie along an olivine-plagioclase cotectic, indicating that the liquid compositions were controlled by cotectic crystallization of olivine and plagioclase. These rocks have compositions appropriate for the magmas that produced mafic cumulates in the NILI.

expanded westward and evolved into a north-plunging trough. Layers of silicic cumulates occur at several levels (Fig. 1). Mafic layers resting on them are marked by a strongly chilled and pillowed base that records replenishment of basic magma into the chamber when it was floored by silicic magma. Silicic cumulates on the walls of the chamber (Fig. 1) have sharp outer (basal) boundaries with gabbroic wall cumulates; this abrupt change to silicic cumulates must record the entry of new silicic magma into the chamber and the downward displacement of basic magma. As a result of multiple basic and silicic replenishment, the magma chamber was probably compositionally stratified most of the time.

Basic dykes, ranging in thickness from 1 to 10 m, occur at all levels, and are essentially identical in composition to chilled basic rocks in the HS. Granitic dykes range in thickness from 1 to ~100 m. All appear to be feeders to the NILI.

Three large transgressive bodies ('troughs') occur within the NILI (Fig. 1). Their steep sides sharply cut cumulates; their tops are conformable with overlying cumulates. They contain mainly massive, variably chilled gabbroic rocks, but their margins are lined with fine-grained granite up to several metres thick. The boundary between granite and gabbro is marked by by a broad zone (1-20 m thick) of chilled mafic pillows which grades inward to gabbro. These relations indicate that the troughs were initiated as silicic feeders and subsequently became filled with basic magma.

Mafic dykes and chilled pillows have compositions that are appropriate for the liquids that produced the mafic cumulates in the NILI[9]. Their compositional variation suggests dominant control by cotectic crystallization of olivine and plagioclase, the main cumulus minerals in the NILI (Fig. 2). The mafic dykes are more primitive than chilled pillows within the troughs (Fig. 3), and no pillows have primitive compositions. The more primitive chilled liquids (dykes) represent basic magma that replenished the magma chamber; the more fractionated chilled liquids (trough pillows) represent magma that evolved within the chamber. The fine-grained granites in the dikes and troughs approach minimum melt compositions. Their compositions are appropriate for magmas that produced silicic cumulates in the HS.

Sequences of layers along the steep walls of the HS record evidence for a major replenishment of silicic magma. Gabbroic cumulates along the western wall pass abruptly inwards to a thick cumulate layer of two-pyroxene quartz monzonite (X in Fig. 1). Further in, chilled mafic rock occurs in sharp contact with and includes rounded masses of the quartz monzonite. The mafic rock coarsens inward and acquires cumulate textures. A thinner but similar sequence occurs in the eastern wall (Fig. 1).

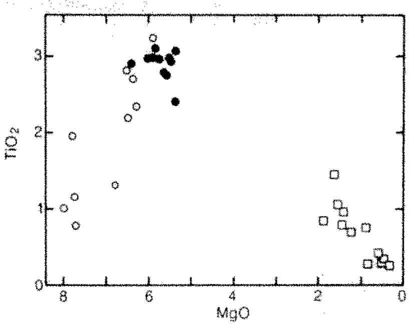

Fig. 3 Plot of MgO against TiO_2 for chilled basic pillows from trough C (●), basic dykes (○) and fine-grained granitic rocks (□), including both dykes and the matrix to basic pillows in troughs. All rocks plotted have compositions that closely approximate liquids related to the NILI.

This sequence of layers developed in the following manner. First, a basic magma filled the chamber to produce the outer mafic cumulates. This magma was suddenly displaced downward (because of its higher density) by a replenishment of silicic magma which produced the silicic wall cumulates. A later replenishment of basic magma displaced this silicic magma upward, engulfed some silicic wall cumulates, and produced the inner mafic cumulates.

The downward displacement of basic magma by silicic magma can be explained readily if that event corresponds to the silicic influx related to the formation of trough C (Fig. 1) and if, as the compositions of chilled pillows in the trough suggest, the gabbroic rock in the trough crystallized from collapsed resident basic magma. Silicic cumulates at lower level (Y in Fig. 1) can be similarly related to trough B.

The formation and expansion of trough C strongly displaced the existing cumulates and caused a major expansion of the magma chamber (Fig. 1). The abrupt inward transition along the walls from basic to silicic cumulates and the lack of evidence for multiple silicic injections within trough C suggest that silicic replenishment occurred in a single pulse. The size of the trough and the great contrast in densities between resident basic and new silicic magmas suggest that emplacement and collapse occurred rapidly, probably on the scale of hours.

These relations demonstrate that a basic magma chamber was invaded from below by a large volume of silicic magma which was emplaced along fractures in the existing cumulates. Although there are not enough data to specify a unique three-dimensional solution to the disruption of the chamber and, therefore, to the volume of new silicic magma, it is possible to present a reasonable solution in two dimensions based on the presently exposed section of the intrusion. Figure 4 provides three such sections, which trace the evolution of the chamber from before the formation of trough C to after a succeeding replenishment of basic magma. The new silicic magma shown in Fig. 4*b* covers an area of ~20 km^2. Assuming a minimum mean thickness of 1 km, at least 20 km^3 of new silicic magma was emplaced at the time of trough formation. This estimate is conservative because, with the roof removed, more silicic magma may have existed at higher levels of the chamber or erupted from the chamber. Figure 4*c* illustrates the state of the magma chamber after some side-wall crystallization of silicic magna and further replenishment of basic magma.

The rapid upward movement of silicic magma, as recorded in the NILI, offers an attractive alternative to some current interpretations of explosive silicic volcanism and occurrences of magma mixing. Indirect evidence of rapid movement of silicic magma from depth has been noted in some recent studies[10,11,12]. Large volumes of silicic magna may have risen rapidly from depths of 25-30 km to erupt to form the Fish Canyon tuff[10]. At least 27 km^3 of rhyolite magma evolved and segregated in 15,000 yr beneath Santorini[11]. This amount of magma could not

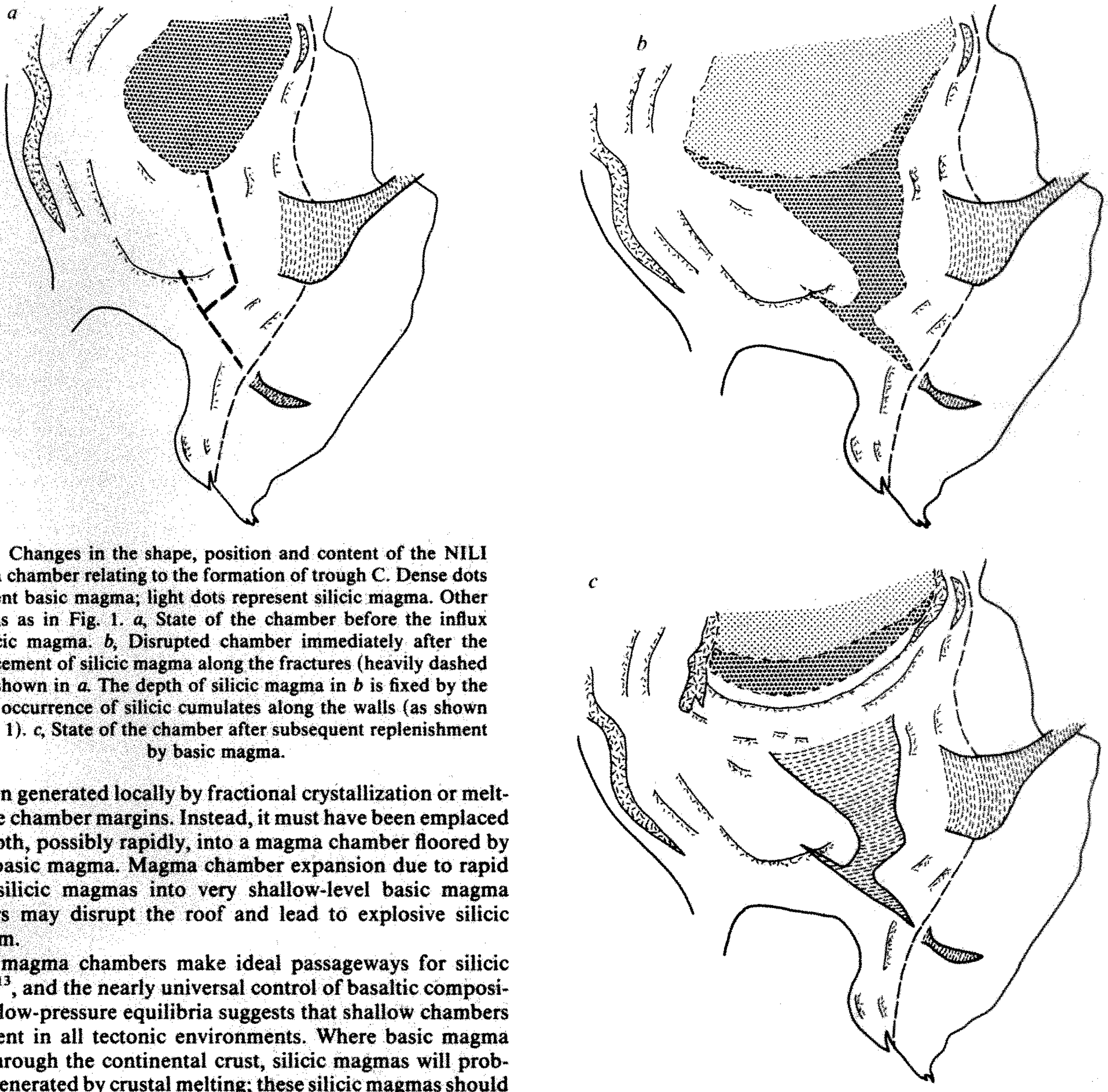

Fig. 4 Changes in the shape, position and content of the NILI magma chamber relating to the formation of trough C. Dense dots represent basic magma; light dots represent silicic magma. Other patterns as in Fig. 1. *a*, State of the chamber before the influx of silicic magma. *b*, Disrupted chamber immediately after the emplacement of silicic magma along the fractures (heavily dashed lines) shown in *a*. The depth of silicic magma in *b* is fixed by the lowest occurrence of silicic cumulates along the walls (as shown by Fig. 1). *c*, State of the chamber after subsequent replenishment by basic magma.

have been generated locally by fractional crystallization or melting of the chamber margins. Instead, it must have been emplaced from depth, possibly rapidly, into a magma chamber floored by a more basic magma. Magma chamber expansion due to rapid rise of silicic magmas into very shallow-level basic magma chambers may disrupt the roof and lead to explosive silicic volcanism.

Basic magma chambers make ideal passageways for silicic magmas[13], and the nearly universal control of basaltic compositions by low-pressure equilibria suggests that shallow chambers are present in all tectonic environments. Where basic magma passes through the continental crust, silicic magmas will probably be generated by crustal melting; these silicic magmas should continue to rise from the lower crust after basic magma chambers have been established in the upper crust. They will tend to rise along existing structural discontinuities and pre-heated passageways and are therefore likely to encounter chambers of basic magma in the upper crust. Because of the higher density and temperature of basic magma, silicic magmas will move rapidly to the top of any chamber, acquiring both heat and kinetic energy for further eruption. Any connecting silicic chambers or passageways that lie beneath the basic magma chamber should be tapped rapidly and efficiently and could result in crustal readjustments similar to that shown by the NILI. Entry of silicic magma into a basic magma chamber provides an ideal opportunity to superheat the silicic magma[1], as it can acquire heat both from the basic magma and from the pre-heated chamber margins.

Emplacement of silicic magma into a basic magma chamber is more likely to promote extensive commingling than if basic magma is emplaced into silicic: the density contrast of the two magmas guarantees rapid movement of silicic magma through the basic magma and extensive mutual contact of the most contrasted magmas, even if the magma chamber is compositionally stratified. Very little mixing may result when basic magmas are injected into silicic chambers[14]; if the chamber is compositionally stratified, there can be little chance of mixing between highly contrasted magmas.

Sparks *et al.*[1] recognized that the injection of basic magma into a silicic chamber would provide an effective mechanism for triggering acid explosive eruptions. Because more energy is efficiently transferred to the silicic magma, the opposite sequence, as recognized in the NILI, provides an even more effective setting in which to trigger explosive eruptions and can more readily account for commingling between highly contrasted magmas just before eruption.

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Dutch elm disease as an analogue of Neolithic elm decline

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The decline in elm pollen about 5,000 radiocarbon years ago, recorded in the lake sediments and peats of north-west Europe[1], has long been a source of speculation for palaeoecologists. Initially it was explained as a consequence of climatic change or degrading soils[2]. Later it was put down to the selective removal of elm branches by Neolithic cultures for use as fodder for animals kept in stalls[3–5]. An alternative explanation involves disease[6–8] and this possibility has received increasing support as a result of the recent effects of *Ceratocystis* on elms in Europe[9]. In North America, Davis[10] has examined the possibility of using recent evidence from the decline of *Castanea* this century[11,12] to interpret the decline in *Tsuga* pollen 4,800 years ago. In Europe, the current elm disease has provided an opportunity to observe the effects of the population decline of elms upon the pollen record. Pollen analysis of humus profiles from a woodland once rich in elm show an elm pollen decline of similar proportions to that found in Neolithic levels and also show an increased input of pollen from weed species as a result of the opening of the woodland canopy.

Much of the Lower Greensand ridge running parallel with and to the south of the North Downs in Kent is covered with mixed deciduous forest. At Scords Wood near Westerham (grid reference TQ478522), the oak-dominated woodland of the escarpment was rich in elm (*Ulmus minor* Miller) on the lower slopes. Before the arrival of elm disease at the site in 1978 the pollen rain from this mixed forest consisted of between 5% and 60% *Ulmus* pollen depending upon the local forest composition. All adult trees of elm were affected by the disease and had been killed within about four years. In late 1985, the raw surface humus of the forest floor was sampled by removing successive layers of organic matter 0.5 cm thick. From these the pollen was extracted by conventional methods[13] and the results are shown in Fig. 1.

The diagram is divided into three zones on the basis of the elm pollen content. In the basal zone, SW1, elm pollen levels are in excess of 10%, reflecting conditions before the incidence of elm disease in 1978. There follows a zone in which elm pollen falls progressively, denoting the advancement of the disease and a consequent reduction in flowering even while the trees still lived. Finally, in zone SW3 the elm pollen dies away altogether.

The pollen proportions of the other tree and shrub species (Fig. 1*a*) are unaffected by the elm decline, as are some local woodland types, such as *Mercurialis*, *Urtica* and Filicales (Fig. 1*b*). But several others show an increase during or after the elm fall. Some of these, such as *Allium* and *Rubus*, are also local species of the woodland floor and their increased pollen input is likely to be a consequence of more prolific flowering following the opening of the tree canopy. But many others, such as *Plantago lanceolata*, *Bidens* type and *Campanula* are not present in the woodland and must be entering the pollen rain from more distant habitats. This has occurred because the more open tree canopy has permitted a better dispersal of these pollen types. Overall, there is a marked increase in the richness and diversity of the pollen assemblage after the elm decline. The mean number of pollen taxa recorded in each zone rises from 24 in SW1, through 27 in SW2, to 35 in SW3.

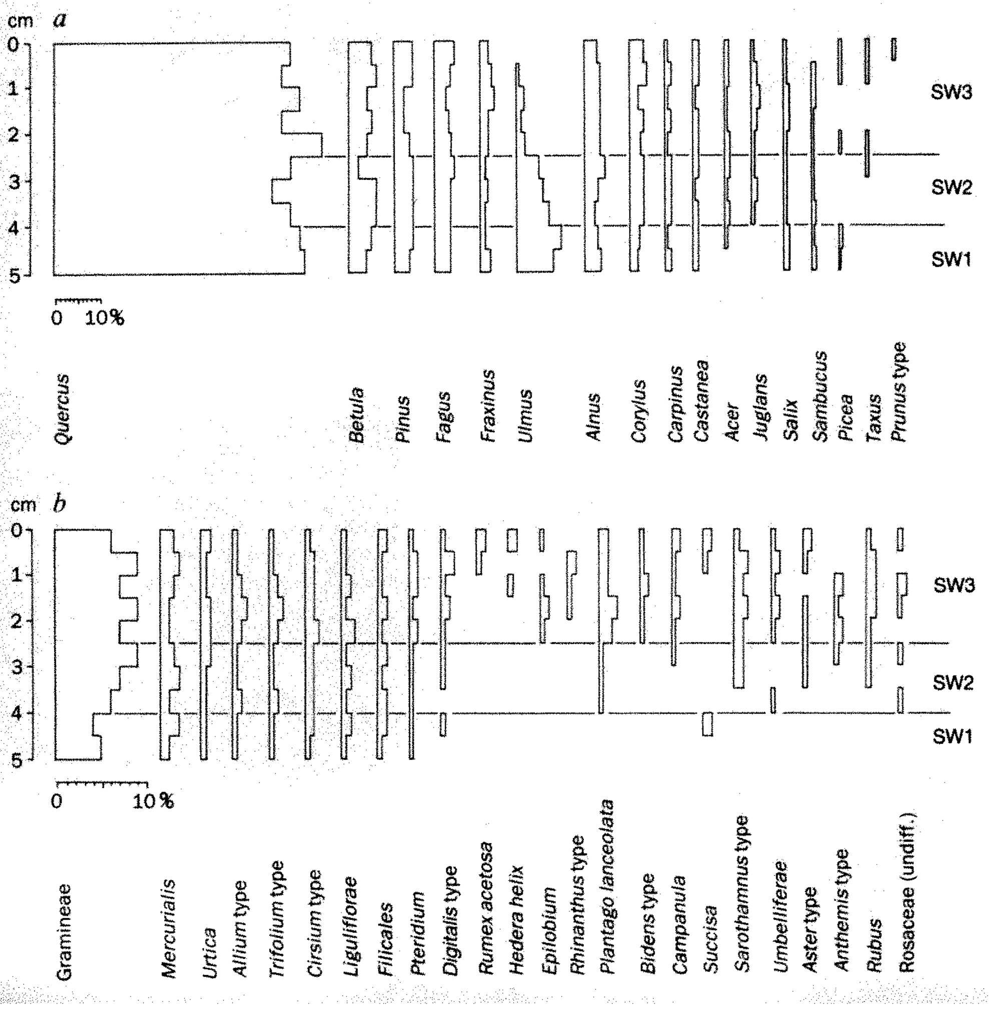

Fig. 1 Pollen diagram from the upper layers of mor humus in deciduous woodland at Scords Wood, Kent. *a*, Tree species; *b*, other species. Data are expressed as percentage of total pollen. The boundary between zones SW1 and SW2 is considered to correspond with the arrival of Dutch elm disease at the site in 1978.

These results are of considerable interest for the interpretation of the Neolithic elm decline. First, they show that the death of elm trees by disease results in a very similar proportional reduction in elm pollen to that found in fossil samples. Second, they indicate that the increased diversity of fossil pollen types at the time of the elm decline, particularly those types often associated with human disturbance, such as *Plantago laceolata*, may come about as a result of the alteration of those physical conditions, such as tree canopy structure, which previously restricted their pollen dispersal.

That weed pollen first comes into prominence in fossil pollen diagrams at the Neolithic elm decline could therefore be a consequence of the loss of elm trees and the resulting general effect on pollen dispersion. Evidence for forest clearance and cultivation before the elm decline is becoming increasingly strong and fits in well with this hypothesis[14,15]. This possibility also weakens the case for a man-induced elm decline, which is based on just such circumstantial evidence. The argument for a disease-induced elm decline in Neolithic times (once strongly rejected by Heybroek[16]) has been strengthened by the historical and dedrochronological work of Rackham. It has also been given greater credence by the recent finding of the beetle vector in Neolithic deposits in Hampstead, London[17], although one beetle does not prove the existence of the disease. The data presented here, however, show that an elm disease could generate pollen changes consistent with those observed in fossil pollen diagrams.

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Ligand binding to the β-adrenergic receptor involves its rhodopsin-like core

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Recently the genes for several hormone receptors that interact with guanine nucleotide binding proteins (G proteins) have been cloned, including the hamster β_2-adrenergic receptor (β_2AR)[1], a human βAR[2], the turkey erythrocyte βAR[3] and the porcine muscarinic acetylcholine receptor (MAR)[4]. All these receptors share some amino-acid homology with rhodopsin, particularly in 7 hydrophobic stretches of residues that are believed to represent transmembrane helices[5]. To determine whether differences in ligand specificity result from the divergence in the sequences of the hydrophilic regions of these receptors, we have expressed in mammalian cells genes for the wild-type hamster and human βAR proteins, and a series of deletion mutant genes of the hamster β_2AR. The pharmacology of the expressed receptors indicates that most of the hydrophilic residues are not directly involved in the binding of agonists or antagonists to the receptor. In addition, we have identified a mutant receptor that has high agonist affinity but does not couple to adenylate cyclase.

In Fig. 1 the amino-acid sequences of the hamster β_2AR, a human βAR and the turkey βAR, all of which bind catecholamines, are compared with those of the porcine MAR and bovine opsin, which interact with acetylcholine and retinal, respectively. The hamster β_2AR and the turkey βAR both couple to adenylate cyclase but differ in their specificity towards catecholamines[6]; the hamster β_2AR being of the β_2-subclass and the turkey βAR of the β_1-subclass. Previously, the pharmacology of the human receptor was unknown. Each group of receptors modulates different intracellular second messengers through interactions with specific G proteins; G_s for the βARs, G_o and G_i for MAR[7] and transducin for opsin[8]. The different receptors vary in amino-acid sequence mostly in their C-terminal regions and their putative third intracellular segments. To investigate the role of these hydrophilic regions in the binding of ligands by the hamster β_2AR, we constructed the series of deletion mutants listed in Table 1.

The genes for the hamster β_2AR, its human homologue and the deletion mutants were inserted into the SV40-derived vector pSVL as described in Fig. 2, legend. Transfection of the expression vector clones containing either the human or the hamster βAR cDNAs into COS-7 (ref. 9) cells resulted in an increase in the specific binding of the β-adrenergic antagonist iodocyanopindolol (^{125}I-CYP) to plasma membranes prepared from the cells (Fig. 2, legend). The expressed βARs displayed affinities for ^{125}I-CYP of 30–50 pM (see Table 1), approximating that of the naturally occurring β_2AR in control COS-7 cells and equivalent to that reported for purified receptor[6,10]. Agonist competition binding experiments (Fig. 2*a*) revealed that the β-adrenergic ligand binding sites resulting from expression of either the hamster or the human gene were indeed of the β_2-subtype, with the relative affinities for agonists being isoprenaline >adrenaline >noradrenaline, in agreement with published values[10].

The expression of the genes encoding the mutant βARs resulted in increases in both ^{125}I-CYP and isoprenaline binding sites (see Table 1). All the expressed mutant receptors were capable of binding ^{125}I-CYP with wild-type affinities with the exception of HAM(del 274–330) which lacked binding and HAM(del 179–187) which bound antagonist and agonist with reduced affinity. HAM(del 274–330) lacks the region encompassing the transmembrane helices VI and VII and HAM(del 179–187) has a deletion within the third external segment. A deletion at the N terminus, HAM(del 21–30), removal of 65 C-terminal residues, HAM(trun 354), and elimination of the third intracellular region, HAM(del 229–236) and HAM(del 239–272), did not dramatically alter ligand binding.

Membrane proteins were electrophoresed and immunoblotted with antisera raised against a peptide corresponding to the C-terminal sequence of the hamster β_2AR protein (Fig. 2, legend). For cells transfected with the wild-type hamster β_2AR gene (plasmid pSVHAM) a strongly reactive band with an apparent relative molecular mass of 67,000 (M_r 67K) was observed which comigrated with purified β_2AR (Fig. 2*b*). A similar immunoreactive band was barely visible for cells transfected with the plasmid pSVL alone, corresponding to the endogenous COS-7 cell β_2AR. In addition to the 67K band,

Table 1 Ligand binding to mutant β_2AR proteins

Protein	Region	COS-7 cells			L cells					
		^{125}I-CYP		Isoprenaline	^{125}I-CYP		Isoprenaline		Adenylate cyclase	
		B_{max} (fmol mg^{-1})	K_D(pM)	EC$_{50}$(nM)	B_{max} (fmol mg^{-1})	K_D(pM)	$R_H:R_L$	K_D(H): K_D(L)(nM)	K_{act}(nM)	Stimulation
Control		48	20	ND	0	—	—	—	—	0 (5)
HUM		2,000	50	300	ND	ND	ND	ND	ND	ND
HAM		3,300	45	300	110	70	55:45	1:50	20	3-6 (6)
HAM(del 21-30)	O1 (N)	2,200	60	300	510	ND	45:55	7:160	10	2 (1)
HAM(del 179-187)	O3	4,400	600	10,000	610	430	55:45	60:7,100	220	5 (1)
HAM(del 229-236)	i3	3,100	60	100	840	ND	60:40	8:190	5	3 (1)
HAM(del 239-272)	i3	3,100	30	10	200	10	100:0	6	—	0 (3)
HAM(del 250-259)	i3	1,600	ND	ND	180	ND	70:30	3:190	30	3 (1)
HAM(del 259-262)	i3	4,100	ND	ND	130	ND	70:30	1:190	50	3 (1)
HAM(del 274-330)	VI-VII	70	ND	ND	ND	ND	ND	ND	ND	ND
HAM(del 343-348)	i4	3,000	ND	ND	73	ND	55:45	1:70	40	4 (1)
HAM(trun 354)	i4 (C)	1.300	50	300	350	150	70:30	2:210	10	2-6 (5)

Membranes were prepared from COS-7 cells and L cells expressing wild-type or mutant βAR, and ligand-binding and adenylate cyclase assays were performed as in Figs 2 and 3. Names of mutant proteins are as in Fig. 2, and of the regions deleted as in Fig. 1. Values of K_D were determined for ^{125}I-CYP binding by Scatchard analysis[17] based on a single class of binding sites. Where no K_D is shown, the density of binding sites was determined in the presence of a single concentration of 225 pM ^{125}I-CYP. In all cases, nonspecific binding was <5%. Measurements of isoprenaline binding and of adenylate cyclase stimulation were as in Fig. 3. For COS-7 cell membranes EC$_{50}$ values were obtained by visual inspection of binding isotherms. For L cells, competition binding data were analysed using the LIGAND nonlinear regression programs of Munson and Rodard[18]. Results are reported for two affinity states of the receptor only in cases where a two-site fit was significantly better than a one-site fit ($P<0.01$). Adenylate cyclase stimulation parameters were determined by nonlinear regression analysis. The fold-stimulation by adenylate cyclase is given as a range in cases where more than one clonal isolate expressing the same mutant βAR was studied. The number in parentheses indicates the number of clonal isolates of each mutant characterized. All data shown are the means of 1-5 experiments, each done in triplicate. Standard errors were <15%. Abbreviations: N, N-terminus; C, C-terminus; ND, not determined; EC$_{50}$, value at 50% maximal binding; B_{max}, saturation value for ^{125}I-CYP binding to membranes; $R_H:R_L$, ratio of receptors in the high- and low-affinity states.

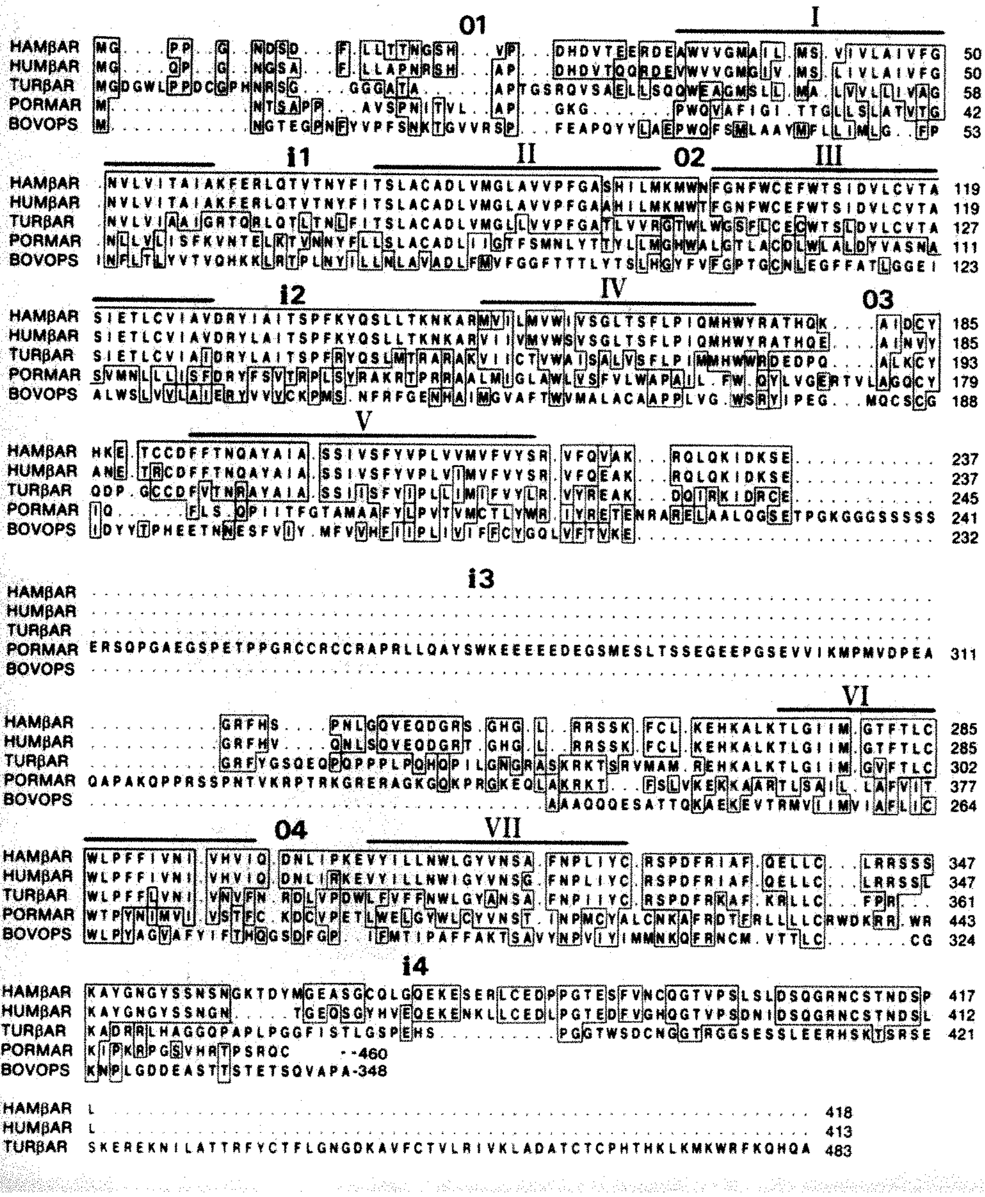

Fig. 1 Comparison of amino-acid sequences for G-protein coupled hormone receptors and rhodopsin. The amino-acid sequences for the hamster β_2-adrenergic receptor (HAMβAR)[1], human β-adrenergic receptor (HUMβAR)[2], turkey β-adrenergic receptor (TURβAR)[3], porcine cerebral muscarinic acetylcholine receptor (PORMAR)[4], and bovine opsin (BOVOPS)[5] are shown. The entire sequence for each receptor is shown with gaps (·) introduced to obtain the best alignment. Amino acids have been aligned to display regions of maximum homology among all the sequences, with identities indicated by boxes around individual amino acids. Regions I-VII correspond to hydrophobic residues postulated to be transmembrane helicies. Based on the rhodopsin model the hydrophilic residues between each transmembrane helix are designated extracellular (O) or intracellular (i) to indicate the proposed orientation of each segment of the protein with respect to the membrane[19].

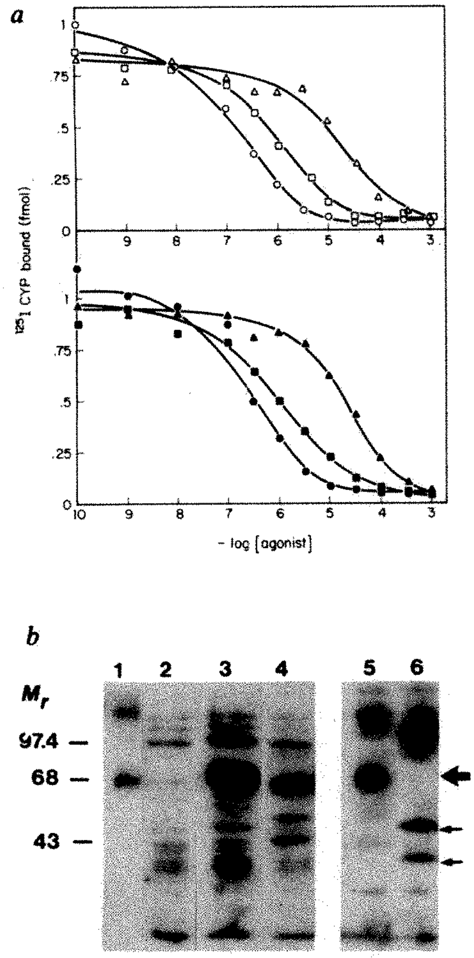

Fig. 2 Expression of wild-type and mutant βAR in COS-7 cells. *a*, Competition for the binding of ^{125}I-CYP by (−)isoprenaline (○), (−)adrenaline (□), and (−) noradrenaline (△) to membranes from COS-7 cells transfected with pSVHAM (open symbols, above) or pSVHUM (closed symbols, below). Total ^{125}I-CYP binding at saturation was 3,300 fmol per mg protein (pSVHAM) and 2,000 fmol mg^{-1} (pSVHUM). Control COS-7 cell transfected with pSVL had 48 fmol per mg protein ^{125}I-CYP binding sites. The data shown are a representative example of three separate experiments, each point performed in duplicate. Nonspecific binding of ^{125}I-CYP in the presence of 10 μM alprenolol was $<5\%$. *b*, Protein immunoblot of βAR-related proteins expressed in cells transfected with pSVL (lanes 2), pSVHAM (lanes 3, 5), pSVHAM(del 239–272) (lane 4) and pSVHAM(del 274–330) (lane 6). Lane 1 contains purified hamster lung β_2AR. Numbers at left, size standards in thousands; large arrow, position of wild-type β_2AR; small arrows, positions of mutant proteins.

Methods. Mutations were introduced into the hamster β_2AR gene (contained in pSVHAM) by oligonucleotide-directed mutagenesis[20]. Deletions were made using oligonucleotides 30 nucleotides (nt) in length containing sequences evenly flanking the desired deletion. These mutants are designated as pSVHAM(del *A*–*B*), where *A* and *B* are the first and last amino acid of the hamster β_2AR sequence included in the deleted region. Single base substitutions were introduced using appropriate mismatched oligonucleotides 16–20 nt in length. The mutant HAM(trun 354) contains a single base substitution converting Tyr 354 to a termination codon. Authenticity was confirmed by dideoxy sequencing[21]. The plasmid pSVL contains the SV40 early promoter and the 16S intron derived from the Okayama–Berg vector pL[22] cloned[23] into the *Hin*dIII and *Pst*I sites of pUC13. The hamster β_2AR cDNA and the deletion mutants were cloned between the *Sma*I and *Eco*RI sites of pSVL as a 1.9-kb fragment originating at the *Eag*I site 86 bp 5′ to the initiator Met and extending 23 bp 3′ to the polyadenylation signal, giving pSVHAM. Similarly, cDNA for the human βAR was cloned into the same sites of pSVL as a 1.8-kb fragment beginning at the *Nco*I site corresponding to the initial Met and encompassing the polyadenylation signal to give pSVHUM. Transfection of plasmids into COS-7 cells was carried out using the $CaPO_4$ precipitation method[24]. Plasmid DNA (15 μg) was mixed with 15 μg of calf thymus DNA and precipitated with 125 mM $CaCl_2$. Precipitates were applied to 10^6 COS-7 cells and incubated at 37 °C for 4 h. Cells were glycerol shocked and incubated in fresh media for 72 h, at which time they were collected by scraping and washing once with phosphate-buffered saline. For a rapid determination of ligand-binding properties of various mutants, COS-7 cells expressing the receptor were lysed by freeze-thawing in 15 mM Tris, *p*H 7.5, 2 mM $MgCl_2$, 0.3 mM EDTA. Membranes were pelleted by centrifugation at 40,000*g* and resuspended at 1–3 mg protein per ml in 75 mM Tris, *p*H 7.5, 12.5 mM $MgCl_2$, 0.3 M EDTA. Saturation binding of ^{125}I-CYP (NEN) was measured at a final protein concentration of 20 μg ml^{-1} using 10–400 pM ^{125}I-CYP, with nonspecific binding defined in the presence of 10 μM alprenolol[25]. Isoprenaline binding was measured in competition with 35 pM ^{125}I-CYP, at a final βAR concentration of 5–7 pM, as described[25]. Protein concentrations were determined by the method of Lowry[26]. Protein immunoblot analysis of the membranes was performed as described[1]. Polyclonal antisera raised against a peptide corresponding to the C terminus of the hamster β_2AR protein was used to detect the βAR-specific polypeptides. A peptide having the sequence CLDSQGRN(norleucine)STNDSPL (amino-acid residues 404–418 of the hamster β_2AR) was synthesized using an Applied Biosystems peptide synthesizer and purified by reversed phase HPLC[1]. This peptide was coupled through its N-terminal cysteine to thyroglobulin (R. Mumford and C.D.S., in preparation) and used as an immunogen in rabbits. Total rabbit serum was used as the first antibody in the immunoblot at a 1:500 dilution followed by detection with ^{125}I-protein A as described[1]. Purification of β_2AR was described[10].

specifically immunoreactive proteins with apparent M_r 46K and 55-58K were also observed in the cells transfected with pSVHAM, presumably representing nonglycosylated and partially glycosylated β_2AR proteins, respectively[10]. Immunoreactive bands of higher apparent M_r were occasionally detected and probably represent aggregates of the receptor. HAM(del 239-272), which bound ligand, was expressed at levels similar to that of the wild-type receptor and had the expected M_r. For HAM(del 274-330), which did not bind ligand, immunoreactive peptides of lower M_r were observed. The M_rs of the peptides were consistent with both a deletion in the proteins and a defect in their glycosylation. Whether the lack of ligand binding for this mutant receptor is due to the loss of sequences directly involved in ligand binding or to a defect in protein folding and glycosylation is unknown.

Although the transient expression of the β_2AR genes in COS-7 cells permitted the binding properties of the expressed proteins to be measured rapidly, with minimal selection, the low frequency of expressing cells in combination with the βAR background in this cell line prevented characterization of the coupling of the receptor with the adenylate cyclase system. Consequently, clonal lines of mouse L cells[11] stably expressing the wild-type receptor and mutants which exhibited ligand binding in COS-7 cells, were established by the procedure described in Fig. 3 legend. Mouse L cells were chosen because they have undetectable levels of βAR as assessed by ligand binding (Fig. 3*a*) and they lack a β-adrenergic-stimulated adenylate cyclase, but contain measurable levels of both prostaglandin E_1- and NaF[12]-stimulated adenylate cyclase activity (data not shown). The density of receptor sites for each of the cell lines was measured by ^{125}I-CYP binding (Table 1). Because L cells lack an endogenous βAR background, the K_D values for ^{125}I-CYP and isoprenaline binding to each of the cell lines could be calculated. The affinities for ^{125}I-CYP of wild-type and mutant hamster β_2AR proteins expressed in L cells were similar to those observed in COS-7 cells (Fig. 3*a* and Table 1). In these cells, HAM(del 239-272) had a slightly greater affinity for ^{125}I-CYP than the wild-type receptor and mutant HAM(del 179-187) exhibited the same reduced affinity for ^{125}I-CYP as observed for the COS-7 cells (Fig. 3*a*).

Data for isoprenaline competition binding and stimulation of adenylate cyclase in membranes containing the wild-type and

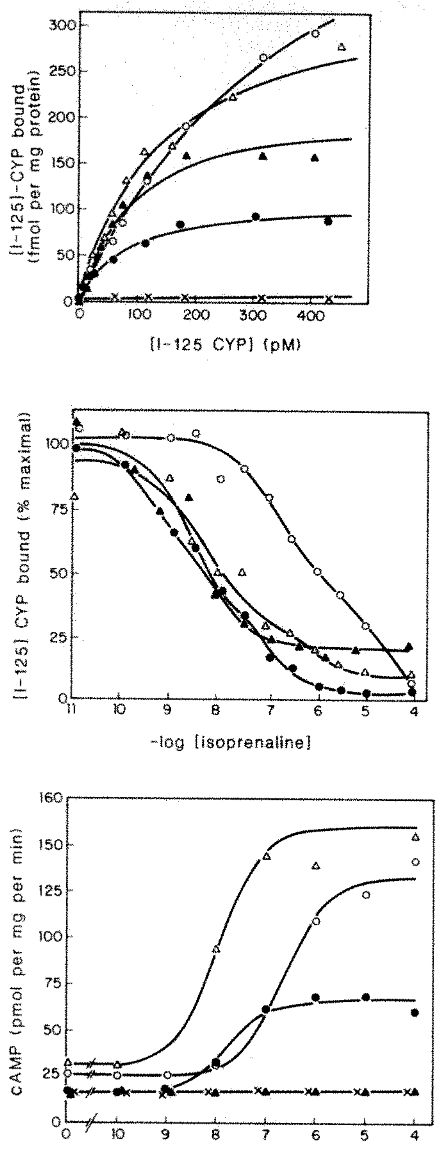

Fig. 3 Saturation binding of ^{125}I-CYP (*a*), isoprenaline competition for ^{125}I-CYP binding (*b*), and isoprenaline stimulation of adenylate cyclase (*c*) in membranes from L cell lines containing pSVL (×), pSVHAM (●), pSVHAM(del 179-187) (○), pSVHAM (del 239-272) (▲), and pSVHAM(trun 354) (△). Adenylate cyclase activity (pmol cAMP per mg per min) in the same membranes stimulated by 10 mM NaF was 46 for pSVL, 87 for pSVHAM, 124 for pSVHAM(del 179-187), 73 for pSVHAM(del 239-272), and 101 for pSVHAM(trun 354). Each experiment shown is a representative of 2-5 experiments for each clone.

Methods. To obtain L cell lines expressing the βAR, each plasmid was cotransfected with 5 μg of pRSVneo[27] as described in Fig. 2. Plasmid-bearing cells were selected by the addition of 500 μg ml^{-1} Geneticin (gentamicin, G-418, Gibco) to the media 24 h posttransfection[28]. Geneticin-resistant colonies (12-24) were picked from each of two separate transfections and assayed for ^{125}I-CYP binding as in Table 1. In all experiments 30-70% of the geneticin-resistant clones co-expressed the βAR. Positive clones (4-10) for each mutant were characterized for ligand binding. Plasma membranes were prepared from L cells by hypotonic lysis in 1 mM Tris, *p*H 7.5. After centrifugation at 40,000*g*, the membranes were resuspended at 1-3 mg protein per ml in 75 mM Tris, *p*H 7.5, 12.5 mM $MgCl_2$, 1.5 mM EDTA. Ligand-binding experiments were performed as described in Fig. 2. Adenylate cyclase stimulation in the presence of isoprenaline or 10 mM NaF was measured as described elsewhere[29].

several of the mutant receptors are shown in Fig. 3*b* and *c*. The wild-type receptor exhibited both high- and low-affinity binding sites for isoprenaline (see Table 1)[13]. The occurrence of two agonist affinity sites has been attributed to an equilibrium between free receptor and receptor complexed with G_s. Membranes prepared from cells expressing the wild-type receptor also demonstrated a dose-dependent activation of adenylate cyclase by isoprenaline and NaF, but membranes derived from the control cells showed stimulation of adenylate cyclase only by NaF. Although the maximum stimulations of adenylate cyclase by isoprenaline varied from 3- to 6-fold for different clonal isolates of each mutant, they were identical to the NaF stimulations in the same membranes (Fig. 3*c*) indicating that the expressed receptors were fully coupled to adenylate cyclase[13,14]. In sharp contrast with the wild-type receptor and HAM(trun 354), HAM(del 239-372) containing membranes exhibited a single high-affinity isoprenaline binding site and did not show any stimulation of adenylate cyclase by isoprenaline (Table 1 and Fig. 3). It is not known whether the affinity exhibited by this site reflects the K_D of the free mutant receptor or of an inactive complex between the receptor and G_s. The lack of adenylate cyclase coupling to this mutant receptor might be due to either the elimination of a site on the receptor for G-protein interaction or to the importance of the deleted region in maintaining an active conformation. HAM(del 179-187), which showed a reduced affinity for isoprenaline, also required higher levels of isoprenaline for cyclase stimulation (Table 1 and Fig. 3). The remaining mutants showed both high- and low-affinity states and stimulated adenylate cyclase by 2-8-fold in an isoprenaline-dependent manner (Table 1). The variation in the absolute level of stimulation between different clonal isolates allows for only a qualitative analysis of receptor-cyclase coupling. Future experiments measuring the stimulation of the GTPase of G_s[14] or the effect of GTP analogues on hormone binding will be necessary to compare quantitatively the abilities of the mutant receptors to couple with G proteins.

The absence of a large hydrophilic domain involved in ligand binding implies that ligands interact either with a site located close to the membrane or within the hydrophobic core of the protein. This hypothesis is supported by the observation that in the case of the turkey βAR, an affinity label bound to the receptor can covalently react with associated glycolipids[15]. Previously we had proposed, on the basis of the homology between the hamster β_2AR and bovine opsin that the β-adrenergic ligands interact with the receptor in a manner analogous to that of retinal interacting with opsin[1]. In rhodopsin retinal binds within the hydrophobic transmembrane region of the protein rather than to external hydrophilic residues[16]. The results described here lend further support to this model.

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The condensing vacuole of exocrine cells is more acidic than the mature secretory vesicle

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A number of intracellular, membrane-bound compartments in both the endocytic and exocytic pathways of eukaryotic cells have an acidic internal *p*H[1]. In endocrine cells, the mature secretory vesicle has an acidic *p*H; secretory vesicles isolated from exocrine cells, however, appear to have a neutral *p*H[2]. Recently we have used a newly developed immunocytochemical technique[3] to map low-*p*H compartments in insulin-secreting islet cells with the electron microscope and find that during the maturation of the secretory vesicle there is a progressive acidification of these vesicles that begins as soon as the *trans* Golgi condensing vacuoles form[4]. Now we have used this technique to examine two exocrine cells: the pancreatic acinar cell and the parotid serous cell. In both cell types, the *trans* Golgi condensing vacuoles are acidic and accumulate the low-*p*H probe to the same extent as condensing vacuoles of insulin-secreting islet cells. Unlike insulin-secreting cells, however, maturation of the granules is accompanied by a return of luminal *p*H to near neutrality. Therefore, although the *p*H of storage granules in exocrine and endocrine cells is different, the *p*H of the condensing vacuoles in both cells is acidic.

DAMP (3-(2,4-dinitroanilino)-3′-amino-*N*-methyldipropylamine) accumulates in acidic compartments and can be localized in plastic-embedded tissue sections with an antibody against dinitrophenol (DNP)[5]. To visualize acidic compartments in exocrine pancreatic cells by indirect immunofluorescence, isolated acini were incubated with 30 μM DAMP, fixed and embedded in Epon. Serial thick sections (0.5 μm thick) were prepared; one section was incubated with anti-amylase serum to localize secretory granules and the adjacent section was incubated with anti-DNP immunoglobulin G (IgG) to localize acidic compartments. DAMP was located in vacuoles that were scattered in the cytoplasm of each cell (Fig. 1*a*). In the companion section that was processed to localize amylase, there were numerous brightly fluorescent granules clustered together in the apex of the cells (Fig. 1*b*). Comparing the two images reveals that most of the amylase-positive granules were not positive for DAMP. Only a few amylase-positive granules just above the nucleus contained DAMP. Therefore, only a subpopulation of the amylase-positive granules appeared to be acidic.

Both amylase and DAMP can be localized in Epon thin sections by an indirect protein A-gold procedure. Figure 1*c* and *d* shows the same juxtanuclear region of an exocrine cell from an acinus examined on adjacent serial thin section (80–100 nm thick) for the presence of DAMP (Fig. 1*c*) and amylase (Fig. 1*d*). The density of anti-amylase serum binding sites in the proteinaceous core of both condensing vacuoles and mature granules was the same. In contrast, only the proteinaceous core of the condensing vacuoles had a high density of anti-DNP IgG binding sites.

These qualitative results were confirmed by a quantitative analysis of the distribution of DAMP-specific gold particles in various cellular compartments of the exocrine cell (Table 1). The density of anti-DNP IgG binding sites over the mature secretory vesicles was nearly the same as the density over the nucleus, which indicates these vesicles were not acidic. In contrast, the density of gold particles in the condensing vacuoles was, on average, ninefold greater than in the mature vesicles. The density of DAMP-specific gold particles in lysosomes was higher than in the condensing vacuole. All anti-DNP IgG binding was abolished if the DAMP-treated acini were post-incubated in the presence of monensin (Table 1). Therefore, in these

Table 1 Density of monoclonal anti-DNP binding sites on pancreatic acinar cells

	Number of gold particles per μm^2 of cell compartment							
	Experiment 1		Experiment 2				Experiment 3	
Cellular compartment			Control		Monensin			
Golgi stack	2±1 *n* = 10	(21.58)	3±1 *n* = 7	(18.41)	1±0.2 *n* = 10	(26.38)	2±1 *n* = 9	(17.87)
Condensing vacuoles	55±9 *n* = 19	(15.42)	53±12 *n* = 11	(10.40)	2±0.6 *n* = 10	(16.29)	60±10 *n* = 11	(9.87)
Granules	6±1 *n* = 14	(19.13)	6±1 *n* = 10	(15.02)	2±1 *n* = 10	(19.13)	7±1 *n* = 10	(15.57)
Lysosomes	111±15 *n* = 15	(6.09)	74±11 *n* = 4	(4.86)	2±1 *n* = 9	(8.93)	99±14 *n* = 9	(10.76)
Nucleus	2±0.3 *n* = 7	(27.12)	3±1 *n* = 5	(27.78)	2±0.3 *n* = 7	(36.21)	1±0.3 *n* = 5	(30.51)

Values are means ± s.e.m.; *n*, no. of pictures evaluated. The no. of μm^2 evaluated for each compartment is shown in parentheses. Dispersed pancreatic acini, prepared as described[14] were incubated in a KRB-HEPES medium containing 0.1% bovine serum albumin (BSA) in the presence of 30 μM DAMP for 30 min at 37 °C (experiment 1). In experiment 2, the cells were incubated an additional 30 min in the presence or absence of 25 μM monensin. In experiment 3, acinar cells were incubated with DAMP for 2 h. At the end of each incubation, cells were rinsed, fixed for 2 h at room temperature with 1% glutaraldehyde buffered with 0.1 M sodium phosphate *p*H 7.4, dehydrated and embedded in Epon. Thin sections were collected on nickel grids and immunostained by the protein A-gold technique[15]. To localize DAMP, sections were incubated for 2 h with monoclonal anti-DNP antibody (0.1 μg IgG ml^{-1}) followed by rabbit anti-mouse IgG (12 μg ml^{-1}) and protein A-gold (1:70 v/v) for 1 h at room temperature. Sections were stained sequentially with uranyl acetate (20 min) and lead citrate (10 min). The distribution of DAMP in cellular compartments was quantified on electron micrographs at a calibrated magnification of 44,100. The number of gold particles and the areas of respective compartments were recorded with an electronic pen on a graphic tablet (Tektronix, type 4933) connected to a microcomputer (IBM PC) programmed to calculate the number of particles per μm^2 of the compartment.

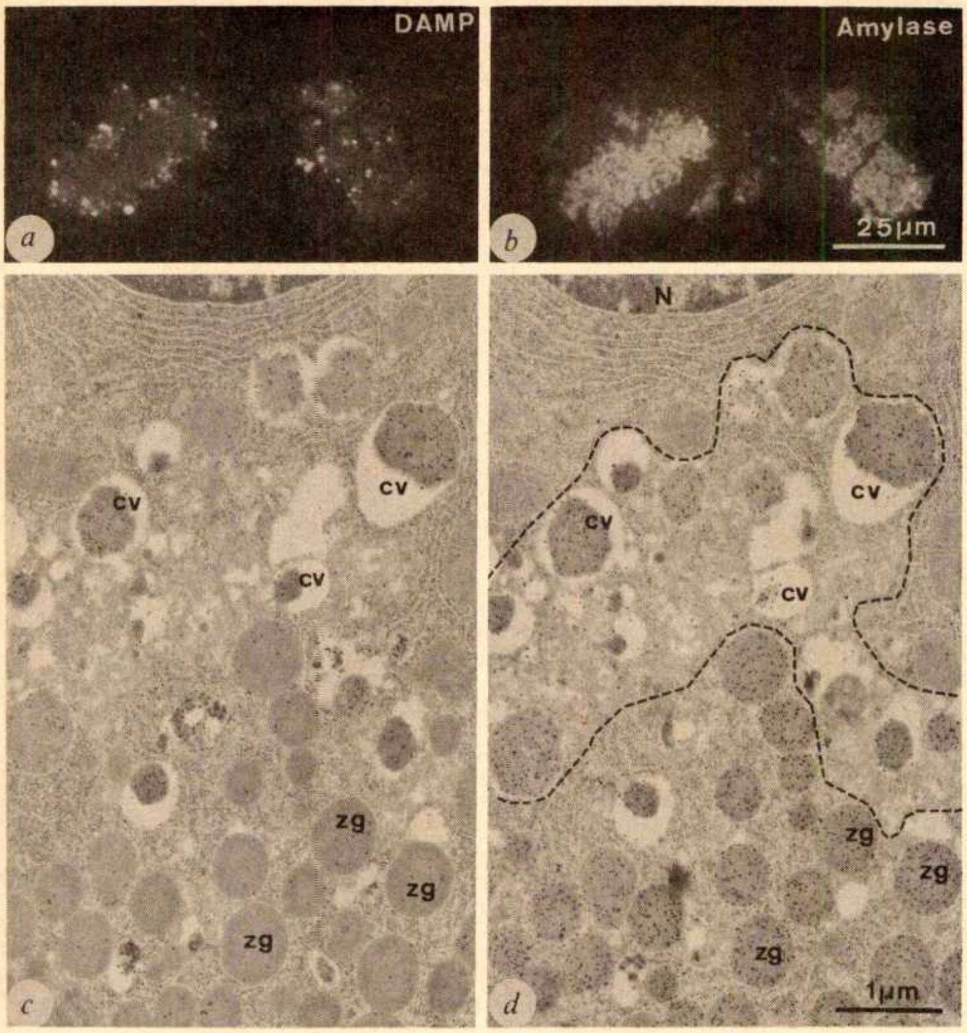

Fig. 1 *a,b*, Localization of DAMP (*a*) and amylase (*b*) by indirect immunofluorescence[16] in consecutive serial sections of two isolated pancreatic acini. *c,d*, Consecutive serial thin sections stained for DAMP (*c*) and amylase (*d*) with the protein A-gold technique (gold particles 15-nm diameter). Dotted line, Golgi region; N, nucleus. DAMP immunoreactivity is high over the secretory content of condensing vacuoles (cv) but not over the mature zymogen granules (zg). Both compartments are labelled with gold particles following treatment with anti-amylase serum. See Table 1 legend for the quantification of DAMP immunostaining and for immunocytochemical procedures.

Methods. In *b*, dispersed pancreatic acini were incubated for 30 min at 37 °C in the presence of 30 μM DAMP as in Table 1. Cells were embedded in Epon. Sections collected on glass slides and processed to remove Epon[17] were incubated with anti-DNP IgG (5 μg ml^{-1}) or guinea pig anti-amylase serum (1:100 v/v) for 2 h and then exposed to FITC-conjugated anti-mouse or anti-guinea pig IgG for 1 h. DAMP immunfluorescence reveals sparse spots predominantly in the middle part of the acinar cell, whereas anti-amylase serum stains densely the centre of the acinus (apex of the cells).

exocrine cells only the condensing vacuoles and lysosomes were acidic.

We next examined the parotid acinar cell, an exocrine cell that has a secretory apparatus similar to the pancreatic exocrine cell. This cell also accumulated DAMP in condensing vacuoles (Fig. 2) but not in mature secretory vesicles. By quantitative analysis (Table 2), the density of DAMP-specific gold particles in condensing vacuoles and lysosomes was found to be similar to that in the same compartments of pancreatic acinar cells. Likewise, the Golgi cisternae and mature secretory vesicles did not have significant numbers of DAMP gold particles.

These two exocrine cells join the insulin-secreting endocrine cell[4] and the cultured human fibroblast[5] as cells that have been analysed with the DAMP technique. All four cell types have in common an acidic *trans* Golgi vesicular compartment. Yet the mature secretory vesicles derived from these condensing vacuoles can have either an acidic (endocrine cell) or neutral (exocrine cell) *p*H. Therefore, acidification of the *trans* Golgi condensing vacuoles must be functionally separate from the acidification of the mature secretory vesicle.

Most probably the acidic compartments of the Golgi apparatus have some function in the movement of proteins through the Golgi[6], their post-translational modification[7] and their sorting into regulated and non-regulated exocytic pathways[8]. Drugs that disrupt *p*H gradients inhibit transit from the *medial* to the *trans* compartments of the Golgi apparatus[9] and shift proteins that are normally secreted by a regulated pathway into a non-regulated pathway[10].

All the endocrine secretory vesicles examined to date have been found to have an acidic interior[1], and the exocrine vesicles studied here are neutral. Exocrine cells have a conspicuous morphological and functional polarity; moreover, a number of physiological[11] and cell biological[12] studies suggest that endocrine cells also express a polarized organization. A fundamental difference between endocrine and exocrine cells is the compartment into which they release their secretory product. Whereas most endocrine cells secrete into an 'advascular' compartment, exocrine cells release their product into an 'abvascular' compartment (lumen of the gland). Perhaps the *p*H of the mature secretory vesicle directly or indirectly controls the polarity of movement towards the different surfaces of the cell: low-*p*H vesicles go to advascular surface and neutral-*p*H vesicles go to

Table 2 Density of monoclonal anti-DNP binding sites on parotid acinar cells

Cellular compartment	No. of gold particles per μm^2
Golgi stack	2 ± 0.4 (26.90) $n = 20$
Condensing vacuoles (core and halo)	73 ± 12 (8.77) $n = 16$
Granules	2 ± 0.4 (39.27) $n = 16$
Lysosomes	159 ± 32 (3.23) $n = 11$
Nucleus	2 ± 0.3 (20.68) $n = 7$

Results are presented as in Table 1.

the opposite surface. In support of this proposal, Caplan *et al.*[13] recently reported that NH_4Cl, a weak base that raises the *p*H of intracellular compartments, abolishes the exclusive secretion of laminin and heparin sulphate proteoglycan from the basolateral surface of polarized MDCK cells.

The alkalinization of the maturing exocrine secretory vesicle clearly indicates that the *p*H of this compartment is regulated, perhaps by the removal of the proton pump from the vesicle membrane. Alternatively, the pump may be inactivated or a permeant anion channel may close[1]. All these possibilities will lead to the dissipation of the *p*H gradient in the condensing vacuole as it matures into a secretory vesicle. Future studies of the exocrine cell may lead to important insights into how *p*H is regulated in various intracellular compartments.

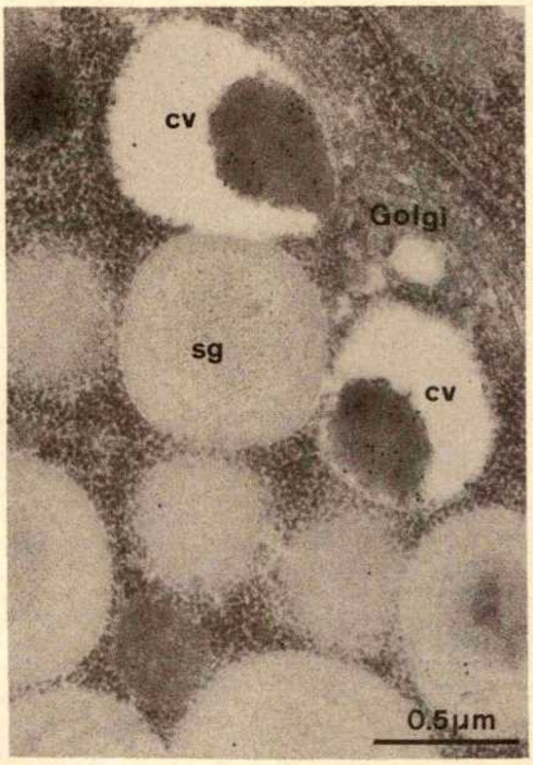

Fig. 2 Localization of DAMP in thin sections of parotid exocrine cell. Gold particles (10 nm diameter) are numerous over condensing vacuoles (cv) but virtually absent over mature secretory granules (sg).

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Differential expression of two distinct T-cell receptors during thymocyte development

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The product of the T-cell receptor (TCR) γ-gene[1] has recently been found to be expressed on a subset of both peripheral cells[2] and thymocytes[3,4]. As an initial approach to understanding the role of this γ-chain of TCR (TCRγ) in T-cell development, we have studied the ontogeny of TCR expression at the protein level in the developing murine thymus. We show here that the first T3-associated TCR to be expressed in the developing thymus is a disulphide-linked heterodimer composed of a γ-chain of relative molecular mass 35,000 (M_r 35K) and a 45K partner (termed TCR δ). This TCR γδ is first detected approximately two days before the appearance of cell-surface TCR αβ heterodimers. We report that *N*-glycosidase digestions reveal that all of the γ-protein expressed on fetal thymocytes, as in adult $CD4^-8^-$ ($L3T4^-$, $Lyt2^-$) thymocytes[4], bear *N*-linked carbohydrate side chains. The major γ-gene transcribed in mature, αβ-bearing T cells ($V_{\gamma1.2}C_\gamma2$) encodes no *N*-linked glycosylation site[1] so these results suggest that the fetal γδ receptor defines a distinct T-cell lineage whose development in the thymus precedes classical αβ-bearing cells.

To characterize the surface CD3(T3)-associated receptor complex, immunoprecipitations were performed on surface radioiodinated fetal thymocytes of different gestational age with an anti-CD3 monoclonal antibody (mAb) (145-2C11)[5,6] and an anti-TCR/CD3 γ antibody[4]. Figure 1 shows the results of immunoprecipitations with the anti-CD3 and anti-γ on radioiodinated fetal thymocytes from gestational days 15, $17\frac{1}{2}$ and 20 (newborn). Samples were electrophoresed on SDS-polyacrylamide gel electrophoresis (PAGE) gels under reducing conditions. On day-15 thymocytes, both the anti-CD3 and anti-γ immunoprecipitated a 35K and a 45K protein. (The murine CD3 components on these cells do not label well using this lactoperoxidase procedure and are thus not easily visualized.) The M_rs of the two precipitated proteins are different from either TCRα or β which range from 37K to 43K as seen with an anti-CD3 precipitation of whole spleen (Fig. 1*a*, lane 5). On day $17\frac{1}{2}$, the anti-γ precipitation shows larger amounts of the same 35K and 45K proteins; however, in addition to these bands, anti-CD3 also precipitates significant amounts of 37–43K material corresponding to the αβ heterodimer. The identity of the 37–43K protein was confirmed by immunoprecipitation with an anti-TCR β-peptide serum (data not shown). By day 20, only the αβ heterodimer is seen in the anti-CD3 immunoprecipitate, although a small amount of the 35K and 45K proteins is present in the anti-γ immunoprecipitate. These results indicate that the first CD3-associated receptor to arise in ontogeny (day 15) is a non-αβ heterodimer containing the γ-chain and a second protein which we term TCRδ in accordance with the nomenclature suggested by Brenner *et al.*[2]. Both the γδ and αβ receptors are present in similar amounts by day $17\frac{1}{2}$ at a time when most thymocytes are $CD4^+8^+$ and 'mature' $CD4^+8^-$ thymocytes have begun to appear[7]. At the time of birth (day 20) the αβ heterodimer is the predominant CD3-associated heterodimer.

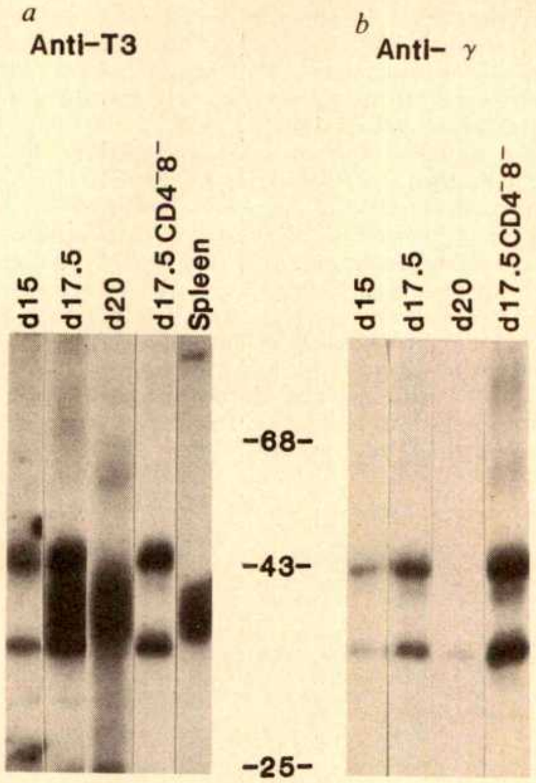

Fig. 1 Differential expression of two distinct T-cell receptors in thymocyte development. Unfractionated fetal thymocytes from day 15, $17\frac{1}{2}$ and 20 (d15, d17.5, d20) of gestation, $CD4^-8^-$ cells from day $17\frac{1}{2}$, and adult spleen cells were surface radioiodinated and lysed in either digitonin or Nonidet-P-40 (NP-40). Lysates were then immunoprecipitated with *a*, anti-T3 or *b*, anti-γ-peptide antibody. Immunoprecipitated proteins were separated on SDS-PAGE gels using reducing conditions. Size markers, M_r in thousands.

Methods. Fetal thymocytes were prepared by mincing fetal thymuses from timed pregnancies. $CD4^-8^-$ day-$17\frac{1}{2}$ fetal thymocytes were prepared by cytotoxic elimination with GK1.5 (rat anti-mouse L3T4) facilitated with MAR 18.5 (mouse anti-rat κ chain) and complement. Cells ($1\text{-}5\times10^7$) were ^{125}I-labelled using lactoperoxidase as previously described[4]. Cells were then lysed in 0.5% NP-40 (for anti-γ precipitations) or 1% digitonin buffer (for anti-T3 immunoprecipitations) containing phenylmethylsulphonyl fluoride, leupeptin, pepstatin and chymostatin. Samples were precleared with rabbit anti-chicken immunoglobulin. Anti-CD3 immunoprecipitations were performed with culture supernatants from the 145-2C11 hybridoma and anti-γ immunoprecipitations were performed with rabbit anti-γ serum[8] affinity purified on a γ-peptide column as described[15]. Immune complexes were collected with protein A agarose beads and run under reducing conditions on 10% SDS-PAGE gels. Gels were dried and labelled and proteins were visualized using Kodak XAR-5 autoradiographic film at −70 °C with enhancing screens.

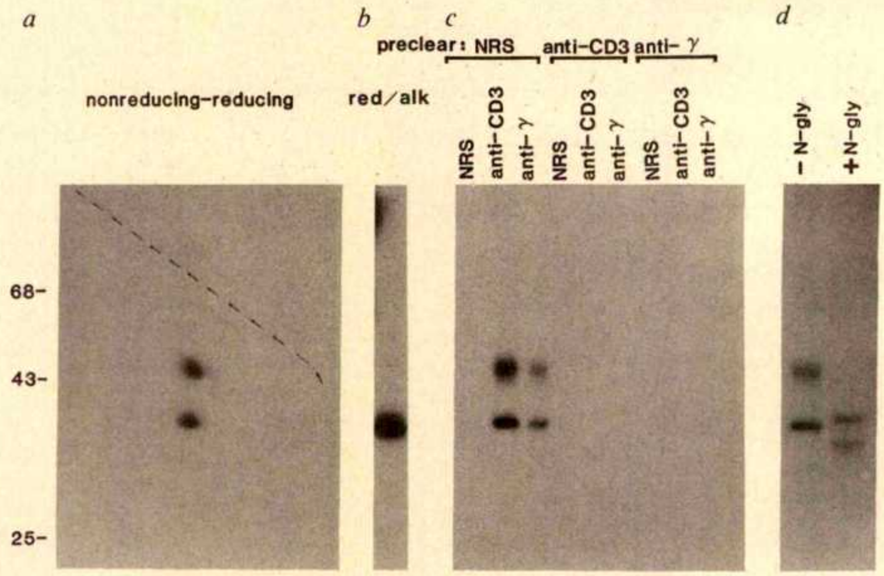

Fig. 2 Biochemical characterization of the TCR γδ in fetal thymocytes. Day-15 fetal thymocytes were radioiodinated and lysed in digitonin as in Fig. 1 and the following biochemical analyses were performed. *a*, Anti-CD3 immunoprecipitates were run without reduction in the first dimension and then under reducing conditions in the second dimension. The diagonal, as determined by the positions of prestained, nondisulphide-linked standards, is shown with the dotted line. Size markers, M_r in thousands. *b*, Anti-CD3 immunoprecipitates were reduced with dithiothreitol, alkylated with iodoacetamide, reimmunoprecipitated with anti-γ and run on a 10% reducing SDS-PAGE gel. *c*, Lysates were precleared by immunoprecipitating with normal rabbit antiserum, anti-γ or anti-CD3. Each precleared sample was split three ways and immunoprecipitated with normal rabbit antiserum, anti-γ or anti-CD3. *d*, Anti-CD3 immunoprecipitates were divided. Half was treated with *N*-glycosidase-F (Genzyme) (lane 2) and half was not (lane 1). After treatment, samples were run on 10% reducing SDS-PAGE gels.

Methods. Cells were isolated, radioiodinated and immunoprecipitated as in Fig. 1. For diagonal gels (nonreducing–reducing), samples were eluted in nonreducing sample buffer and run for 12 h in a 10% SDS-PAGE tube gel followed by a 12.5% reducing slab gel. Reduction-alkylation, sequential immunoprecipitations and *N*-glycosidase-F treatment were performed as described[4,16].

We were able to correlate TCR expression to surface phenotype by examining receptor expression on day-$17\frac{1}{2}$ fetal thymocytes depleted of $CD4^+8^+$ (double positive) and $CD4^+8^-$ (single positive) cells by cytotoxic elimination with GK1.5 (anti-CD4) mAb. As $CD4^-8^+$ cells are not yet present at this gestational age[7], the remaining cells were exclusively $CD4^-8^-$ (data not shown). Immunoprecipitation of this subset with anti-CD3 showed significant amounts of the γδ receptor and little detectable αβ receptor (Fig. 1*a*, lane 4). This result indicates that during development, as in the adult thymus[8], the γδ receptor is found within the double-negative subset whereas the αβ receptor is present predominantly on the single positive and probably double positive subsets.

As with double-negative cells from the adult thymus[4], the γ- and δ-chains in developing murine thymocytes are disulphide-linked. This was demonstrated by performing off-diagonal gels (first dimension under nonreduced conditions, second dimension under reduced conditions) on anti-CD3 precipitates of day-15 fetal thymocytes (Fig. 2*a*). Both the 35K and 45K proteins fall below the diagonal, indicating that they ran as a single disulphide-linked 80K molecule under nonreducing conditions.

To determine which of the two proteins reacted specifically with the anti-γ serum, anti-CD3 precipitates of day-15 thymocytes were reduced (with dithiothreitol) and alkylated (with iodoacetamide) to separate the γ- and δ-chains and then repre-

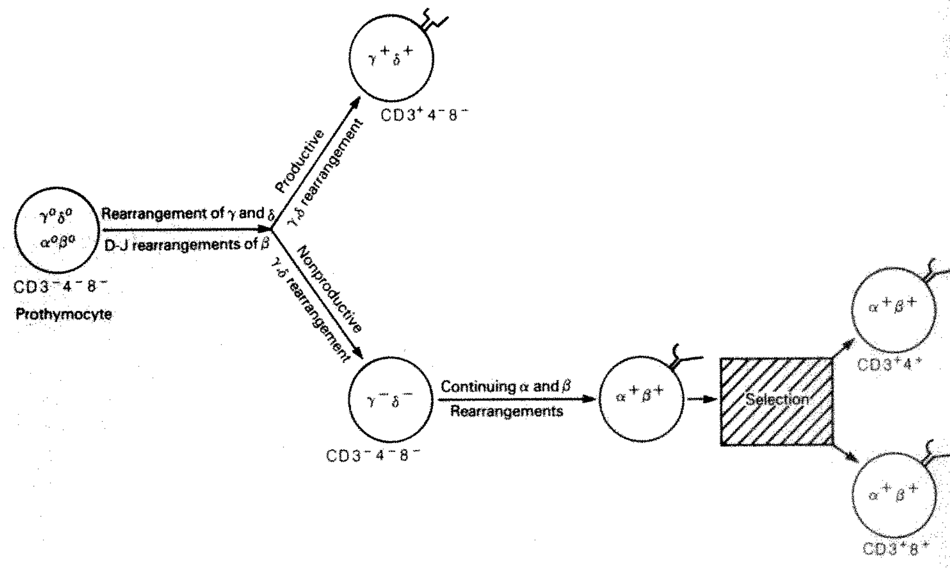

Fig. 3 Model for developmental expression of two distinct T-cell receptors. The model incorporates previous data on TCR gene rearrangement[8,10-12] and our current results on receptor protein expression in developing thymocytes. Synchronous rearrangement of TCR γ- and β-genes is envisioned to occur in prothymocytes on entry into the thymus. Completed rearrangements occur in the γ- and δ-genes at a point when rearrangements of the β-gene are partial. If productive γ and δ rearrangements occur, a CD3-γδ receptor is produced, halting further rearrangements of the β- and α-gene, committing the cell to this lineage. If a nonproductive γ or δ rearrangement occurs, the cell continues to rearrange its α- and β-genes and can become a CD3-αβ bearing cell subject to intrathymic selection.

cipitated with the anti-γ serum (Fig. 2*b*). Under these conditions, only the 35K protein is seen, indicating that this is the γ-chain.

To demonstrate definitively the association of CD3 with the γδ heterodimer, sequential immunoprecipitations were performed on lysates from surface radioiodinated day-15 fetal thymocytes. Figure 2*c* shows that when these lysates were precleared with anti-CD3, all the anti-γ reactive protein was removed. Similarly, when the lysates were precleared with the anti-γ serum, virtually all the detectable CD3-associated receptor chains were removed. These results show that all the surface γ-chain is CD3-associated. Because all of the sequential immunoprecipitations were performed on lysates from freshly isolated cells, we conclude that the surface γ-chain is only present on the small subset of CD3-positive fetal thymocytes despite the fact that most express γ-specific mRNA[6].

Analysis of the *N*-linked glycosylation pattern of the murine γ-chain can distinguish between expression of different γ-gene products. Because no $C_\gamma4$-specific RNA is found in fetal thymocytes[9] and $C_\gamma3$ is a pseudogene[10], the expressed γ-chain on fetal thymocytes could be only derived from $C_\gamma1$ or $C_\gamma2$ (using the nomenclature proposed by Garman *et al.*[11]). $C_\gamma1$ possesses one *N*-linked carbohydrate acceptor sequence[11], whereas $C_\gamma2$ (and $V_\gamma1.2$ to which it rearranges) has no *N*-linked carbohydrate acceptor sites[1,11,12]. Figure 2*d* demonstrates that both the γ- and δ-chains in fetal thymocytes are sensitive to *N*-glycosidase-F, an enzyme which cleaves *N*-linked carbohydrates. The δ-chain reduces to a M_r of 37K and the γ-chain to 32K. The finding that all of the fetal thymic γ-chain is *N*-glycosylated indicates that it is all derived from $C_\gamma1$ rearrangements.

Our demonstration that the γ-chain in freshly isolated populations of developing fetal thymocytes is CD3-associated, disulphide-bonded to a 45K δ-chain and primarily derived from $C_\gamma1$ rearrangements indicates that the cells which bear this receptor early in ontogeny are analogous to the $CD3^+4^-8^-$ cells in the adult thymus, whose γ-chain has recently been shown to have similar biochemical properties[4]. It is difficult to reconcile our findings with models that directly link γ-chain expression on thymic precursors to MHC restriction of αβ-bearing T cells, either through developmental selection of γβ heterodimers[8,13] or reexpression of selected γδ receptors on mature T cells[3,14]. Either of these models predicts that the same γ-chain would be expressed in developing thymocytes as in mature αβ-bearing T cells (that is $C_\gamma2$-$V_\gamma1.2$). The predominant expression of the glycosylated γ species (derived for $C_\gamma1$) in both the fetal and adult thymus of B6 mice instead suggests that the γδ receptor may define a separate T-cell lineage whose intrathymic development precedes that of classic αβ-expressing T cells (Fig. 3). The presence of γ-gene rearrangements in mature cells with the αβ receptor[12] as well as partial β-gene rearrangements in cells that bear the γδ receptor[4] indicates that both lineages may derive from a common precursor. Recent evidence suggests that immature thymocytes begin to rearrange their TCR γ and β genes synchrononously[10]. However, the γ (and possibly δ) locus completes a *V-J-C* rearrangement in most thymocytes by day 15 at a time when many β-locus rearrangements are incomplete (*D-J-C*)[10]. Therefore, as indicated here, the γδ heterodimer is the first T-cell receptor to appear in ontogeny. As thymocytes with the γδ heterodimer predominantly express 1.0-kilobase-gene messenger RNA[4], which is the product of an incomplete *D-J* rearrangement, it is likely that once the product of a productively rearranged γ- and δ-gene is synthesized, further rearrangement of β-(and α-) genes does not occur. If the rearrangement of γ or δ is nonproductive, no full length protein is produced and the cell continues to rearrange its β- and α-genes in an attempt to produce an αβ heterodimer. This model is compatible with the presence of full-length (1.3-kb) β mRNA in $CD3^-4^-8^-$ cells[4,8] and predicts a high frequency of nonproductive $C_\gamma1$ rearrangements in mature αβ-bearing T cells.

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Characterization of murine thymocytes with CD3-associated T-cell receptor structures

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The thymus is the major site for T-cell receptor (TCR) gene rearrangement and T-cell maturation[1,2]. The specific antigen recognition structure (TCR) on murine T cells has been shown to be dependent on a polymorphic set of disulphide-linked heterodimers, containing two integral membrane glycoprotein chains, TCRα and TCRβ, expressed in non-covalent association with an invariant complex of proteins, CD3 (T3)[3–6]. Recently, a novel TCR/CD3 complex, that includes the product of the TCRγ gene, has been identified on a subset of both peripheral cells and thymocytes[7–9]. Here we examine the expression of TCR/CD3 complexes in fetal ontogeny and in the adult thymus. The results demonstrate that CD3$^+$4$^-$8$^-$(T3$^+$, L3T4$^-$, Lyt2$^-$) cells are detected in day-15 fetal thymi, throughout fetal development and in adult thymus. *In situ* hybridization studies indicate that these early CD3$^+$ cells express high levels of TCRγ-specific RNA, low levels of TCRβ-specific RNA and no detectable TCRα-specific RNA. Day-16 CD3$^+$, 4$^-$, 8$^-$ fetal thymocytes can be activated to proliferate and demonstrate cytolytic activity when cultured in the presence of anti-CD3 monoclonal antibodies and interleukin-2 (IL-2). These results suggest that CD3-bearing cells, present early in thymic ontogeny, express a functional TCR and may, therefore, be important in repertoire development.

The availability of a newly derived anti-murine CD3 monoclonal antibody (mAb) specific for the relative molecular mass 25,000 (M_r 25 K) protein component of the TCR complex (designated CD3-ε)[10] has provided a means to study the developing fetal thymus for the presence of CD3$^+$ thymocytes. Flow cytometric (FCM) analysis demonstrated that a subpopulation of CD3$^+$4$^-$8$^-$ fetal thymocytes were first evident on day 15 of gestation (Fig. 1*c–d*) and remained present throughout thymic development (Fig. 1*f, h, j*). On day 18 two additional populations of CD3$^+$ cells were evident. A significant percentage of the CD4$^+$8$^+$ cells were CD3 dull (Fig. 1*i, j*) and in some experiments a small, but significant percentage of CD3 bright, CD4$^+$8$^-$ cells was observed (data not shown). The early CD3$^+$ fetal thymocytes were analysed for TCRα, β, and γ-specific RNA expression by *in situ* hybridization which requires very few cells, examines RNA at the single cell level, and thus shows the population distribution of gene expression. Day-16 fetal

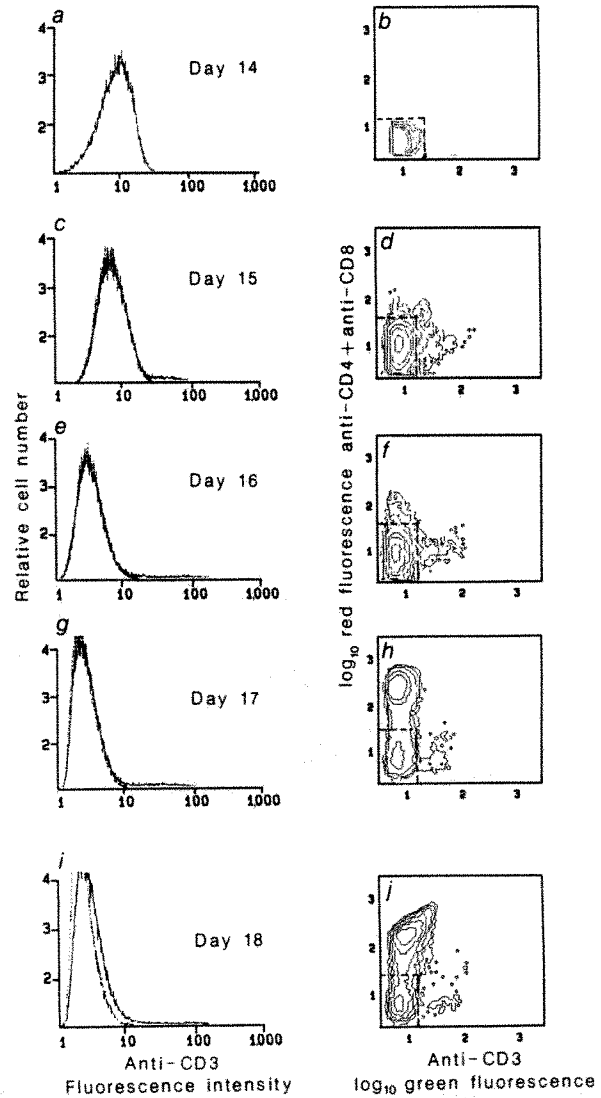

Fig. 1 Multicolour flow cytometry (FCM) analysis of cell-surface CD3 expression in fetal thymus ontogeny. Thymocytes from day-14 (*a, b*), day-15 (*c, d*), day-16 (*e, f*), day-17 (*g, h*) or day-18 (*i, j*) embryos were reacted with the anti-CD3 mAb[20] (solid line, *a, c, e, g, i*) or a control negative hamster mAb (dotted line, *a, c, e, g, i*) followed by FITC-conjugated mouse anti-hamster-immunoglobulin antibody (FITC-MαH). Fetal thymocytes were also reacted with anti-CD3 mAb followed by FITC-MαH followed by biotin-conjugated anti-CD8 (Becton-Dickinson) plus biotin-conjugated anti-CD4 (ref. 21) followed by Texas-Red streptavidin (Bethesda Research) (*b, d, f, h, j*). Immunofluorescence staining and washing were performed as described[10] using aliquots of 1×10^6 cells per tube. All procedures were performed in Hanks balanced salt solution containing 0.1% bovine serum albumin and 0.1% sodium azide. FCM analysis was performed as described[21] using a B-D Dual Laser FACS II equipped with the manufacturer's filters and photomultiplier tubes, an argon ion laser for FITC excitation (488 nm) and a pumped dye (rhodamine 6G) laser for Texas-Red excitation (590 nm) and interfaced to a Digital Equipment Corporation PDP 11/24 computer. All analyses were performed using logarithmic (three decades) amplification and data were collected and analysed using hardware and software designed by the Computer Systems Laboratory of the Division of Computer Research and Technology at the National Institutes of Health. Fluorescence data were collected on 50,000 viable cells as determined by forward light scatter and propidium iodide gating. One colour (FITC) data were displayed as increasing fluorescence intensity (*x*-axis) versus cell number (*y*-axis) (*a, c, e, g, i*). Two-colour data were displayed as contour diagrams (*b, d, f, h, j*), with logarithmically increasing intensities of green (FITC) fluorescence are plottled on the *x*-axis versus logarithmically increasing intensities of red (Texas-Red) fluorescence on the *y*-axis. Constant values of the percentage of total cell number on the *z*-axis were selected to draw the rings or contours around peaks of cells correlating green and red fluorescence. Cells defined to be negative for both red and green fluorescence are shown by boxes in the lower left portion of the contour diagrams. The *x* and *y* coordinates which defined negative cells were selected as the intersection of positive and control negative profiles in each parameter.

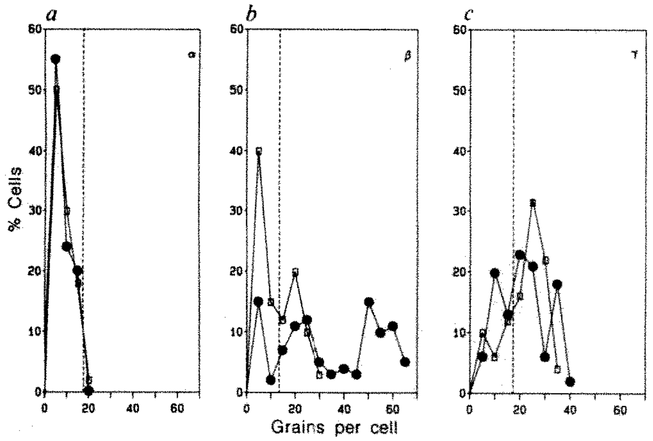

Fig. 2 *In situ* hybridization of RNA in day-16 CD3$^+$ (□) and CD3$^-$ (●) fetal thymocytes. Day-16 fetal thymocytes were reacted with anti-CD3 mAb followed by FITC-MαH. CD3 positive and negative cells were determined by FCM analysis and separated by electronic cell sorting using a Becton-Dickinson FACS II. The CD3$^+$ cells were >97% pure and the CD3$^-$ cells were >99% pure as reanalysed by FCM. Sorted thymocytes were cytospun onto glass slides and fixed with paraformaldehyde in phosphate buffered saline (PBS) and 70% ethanol. *In situ* hybridization was performed with *a*, TCR $C\alpha$; *b*, TCR C_β and *c*, TCR $V_{\gamma1.2}C_{\gamma2}$-specific ^{35}S-labelled RNA probes using a modification of a previously described protocol[22]. Gene inserts were cloned into pGEM1 vectors (Promega Biotec), linearized and transcription was performed with either T7 or SP6 RNA polymerase. Hybridization was performed at 50 °C for 12 h at a concentration of 1×10^8 c.p.m. ml^{-1}. Slides were washed at 54 °C in 2×SSC/50% formamide, followed by incubation with 100 μg ml^{-1} RNase A and 1 μg ml^{-1} RNase T$_1$ for 30 min at 37 °C. Slides were dried and autoradiography was performed with NTB2 photographic emulsion (Kodak). Exposure times were 10 days for TCRα, 5 days for TCRβ and 20 days for TCRγ. Grains were counted on 50–150 cells per slide. Dashed lines represent the maximal number of grains observed in the negative control (RNA sense strand probes). Therefore, points to the right of the dashed lines are considered positive by the limits of this technique.

Table 1 Activation of fetal and adult thymocytes by anti-CD3 mAb

a. Proliferation

		T cell proliferation (c.p.m. × 10^{-3})		
Responder*	IL-2†	medium	145-2C11 (Anti-CD3)	F23.1 (Anti-Vβ8)
B10 Spleen	−	0.4	86.4	1.3
B10 Spleen	+	5.5	164.7	79.8
B10 Fetal thymus	−	0.8	0.7	0.8
(day 14)	+	3.7	6.9	3.3
B10 Fetal thymus	−	0.7	2.6	1.1
(day 16)	+	3.9	68.4	3.1
B10 Adult thymus	−	0.3	1.9	0.5
B10 Adult thymus	+	4.1	13.8	9.3

b. Cytolysis

Effectors‡	Cultured with	mAb added	% Specific lysis§
B10 Adult spleen	—	—	−4.2%
	—	Anti-CD3	28.2%
	Anti-CD3	—	37.9%
	Anti-CD3	Anti-CD3	74.2%
B10 Fetal thymus	—	—	1.1%
(day 16)	—	Anti-CD3	6.8%
	Anti-CD3 + IL-2	—	25.6%
	Anti-CD3 + IL-2	Anti-CD3	58.8%

* 1×10^5 cells were cultured in culture media[10] for 96 h in round-bottom microtitre wells in 7.5% CO$_2$ at 37 °C. The ^{3}H-thymidine (1 μCi) was added to each during the last 16 h of incubation. Radiolabel incorporation was determined as described[10].

† Human recombinant IL-2, 50 units ml^{-1} (Cetus).

‡ 1×10^6 cells were cultured for 7 days as above in the presence or absence of anti-CD3 (50 μl of a high-titred culture supernatant) and IL-2. No activation was observed in the presence of anti-CD3 or IL-2 alone (data not shown).

§ Lysis was assessed on FcR-bearing LPS blast cells as previously described[10]; effector: target ratio, 20:1.

thymocytes were stained with the anti-CD3 mAb and separated into CD3$^+$ and CD3$^-$ subsets by electronic cell sorting. The sorted cells were analysed for TCRα, β, and γ-specific RNA as described elsewhere (D. Pardoll *et al.*, in preparation). As seen in Fig. 2*a*, no significant amount of TCRα RNA was observed in either the CD3$^+$ or CD3$^-$ subpopulations. In contrast, TCRγ RNA was present in most of the CD3$^+$ and CD3$^-$ thymocytes (Fig. 2*c*). Examination of TCRβ RNA showed that among the CD3$^-$ fetal thymocytes ~50% expressed high levels (>40 grains per cell) and ~35% of the cells expressed low levels of TCRβ chain-specific RNA. In contrast, 55% of the CD3$^+$ cells did not express detectable levels of TCRβ RNA and the remainder expressed only low levels (<40 grains per cell) that may represent TCRβ genes that have undergone a *D–J* rearrangement as seen in adult CD3$^+$4$^-$8$^-$ thymocytes[6]. Thus, the majority of the CD3$^+$4$^-$8$^-$ thymocytes express TCRγ RNA but low or undetectable amounts of mature TCRβ and no TCRα-specific RNA. In the accompanying study[11], these CD3$^+$ cells were found to express a second T cell receptor (TCRγδ) associated with the CD3 complex.

CD3$^+$ cells present early in fetal ontogeny were examined for functional activity using the anti-CD3 mAb and the anti-Vβ8 (F23.1) mAb as described[10]. Day-16 fetal thymocytes were harvested and incubated for 5 days with the anti-murine CD3 mAb in the presence or absence of co-stimulating factors. As indicated in Table 1, the anti-CD3 antibody induced significant proliferation of mature peripheral T cells in the absence of exogenous factors. Adult and fetal thymocyte proliferation, however, required exogenous recombinant IL-2. These findings suggest that the requirements for the activation of fetal and adult thymocytes by anti-CD3 mAb may be different than those for mature peripheral T cells. This could be due to differences in the differentiation states of the two populations or could be a result of the presence or absence of another cell population critical for activation. Anti-Vβ8-specific mAb (F23.1) induced the proliferation of adult thymus and peripheral T cells but not day-16 fetal thymocytes, consistent with the absence of F23.1-bearing cells before day 17 of fetal development[13].

Anti-CD3 activated fetal thymocytes were also analysed for cytolytic activity. The anti-CD3 mAb has previously been shown to effectively redirect lysis by murine alloreactive CTL (cytotoxic T lymphocytes) to Fc receptor (FcR)-bearing target cells that do not bear the nominal antigen[10]. As shown in Table 1*b*, anti-CD3 plus IL-2-activated fetal thymocytes reacted with syngeneic lipopolysaccaride (LPS)-induced FcR$^+$ B-cell blasts. The efficient lysis of the LPS blasts depended on the presence of anti-CD3 antibody during the culture period and in the ^{51}Cr-release assay. In several studies lysis of natural killer (NK)-sensitive YAC tumour cells was also observed. This killing was not completely inhibited by anti-FcR antibodies suggesting that anti-CD3-induced cytolytic cells had some NK-like activity. Thus, it would appear that anti-CD3 antibodies induced proliferation and cytolytic activity of CD3$^+$4$^-$8$^-$ fetal thymocytes. Finally, the activated T cells were examined for expression of different differentiation antigens. FCM analysis of cultured day-16 fetal thymocytes showed that the phenotype of the proliferating cells at the time of cytolytic assay was CD3$^+$4$^-$8$^-$ and Vβ8$^-$ consistent with the conclusion that the cytolytic cells are a CD3$^+$, αβ$^-$ T-cell population.

The presence of CD3$^+$4$^-$8$^-$ cells early in fetal ontogeny suggested a possible role for these cells in thymus development

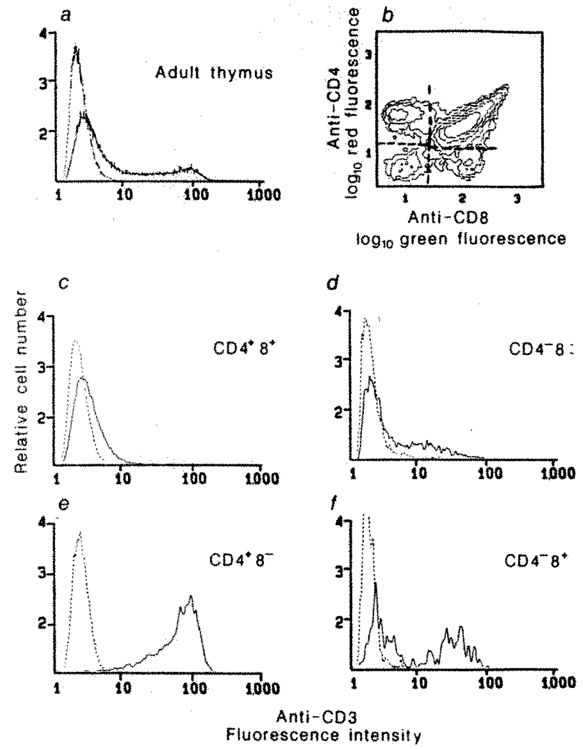

Fig. 3 Three-colour FCM analysis of adult thymocytes. *a*, CD3 expression on whole thymocytes; *b*, CD4 versus CD8 expression on whole adult thymocytes; *c–f*, thymocyte subpopulations as determined by software gating: *c*, $CD4^+8^+$; *d*, $CD4^-8^-$; *e*, $CD4^+8^-$; *f*, $CD4^-8^+$ cells. Solid line, anti-CD3; dotted line, irrelevant hamster mAb followed by FITC-labelled MαH single-parameter green fluorescence profiles (*a*, *c*, *d*, *e*, *f*). After anti-CD3 or control mAb plus FITC-MαH, cells were reacted with biotin-conjugated anti-CD8 and phycoerythrin (PE)-conjugated anti-CD4 (both Becton-Dickinson) followed by Texas-Red streptavidin. List mode data (light scatter, FITC fluorescence, PE fluorescence and Texas-Red fluorescence) were collected on 50,000 viable cells as determined by forward light scatter intensity. *b*, Representative contour diagram of anti-CD8 versus anti-CD4 fluorescence. A similar profile was used to select coordinates for software gating on thymocyte subsets defined by CD8 and CD4. Results of software gating and resulting FITC profiles are shown in panels *c–f*.

during ontogeny and adult life. Therefore, CD3 expression was assessed on the distinct adult thymus subpopulations that have been shown to include dull-$CD5^+$ (Ly-1^+), $CD4^-8^-$ thymocytes capable of differentiating into cortical-type thymocytes *in vitro* and both cortical and mature medullary-type T cells *in vivo*[14,15], $CD4^+8^+$ cortical-type thymocytes that for the most part die within the thymus, and $CD4^+8^-$ and $CD4^-8^+$ mature, medullary thymocytes[15,16]. First, single-colour FCM was performed to assess the expression of CD3 on adult thymocyte populations (Fig. 3). Two distinct $CD3^+$ populations were observed (Fig. 3*a*), a bright $CD3^+$ subset of cells representing 10–15% of total thymocytes and a dull $CD3^+$ subset representing at least 50% of the remaining thymocytes. To discriminate between the thymocyte subsets expressing the CD3 antigen further, adult thymocytes were analysed for coordinate expression of CD8, CD4, and CD3 by three-colour FCM. Figure 3*b* shows that analysis of CD4 and CD8 expression subdivides the thymocytes into four subpopulations ($CD4^-8^-$; $CD4^+8^+$; $CD4^-8^+$; $CD4^+8^-$). As in the fetal ontogeny studies shown above (Fig. 1) and in studies of human adult thymus[17,18], a significant percentage of the $CD4^-8^-$ cells expressed CD3 (Fig. 3*d*). The dull-$CD5^+$, $CD4^-8^-$ subset of these cells have been previously shown to express TCRγδ/CD3 on their cell surface[7,9,11]. The majority of bright-$CD5^+$, $CD4^-8^-$, cells are also $CD3^+$ (J.A.B. and B.J.F., unpublished observation). The TCR genes, expressed by this adult thymocyte population are at present under study. The major thymocyte population bears both CD4 and CD8 antigen and expresses low levels of CD3 (Fig. 3*c*) consistent with the conclusion that at least a portion of the cortical-type thymocytes express TCR/CD3 protein on their cell surface. But the level of CD3 expression was extremely low, suggesting that either very little TCR is expressed on these cells or the cell surface configuration of the TCR/CD3 complex alters the binding of the mAb.

The mature $CD4^+8^-$ cells express the highest levels of CD3 (Fig. 3*e*) similar to the levels of CD3 seen on peripheral $CD4^+8^-$ T cells (J.A.B. *et al.*, unpublished observations). Staining of the $CD4^-8^+$ T cells with the anti-CD3 antibody resulted in the discrimination of two populations, one CD3-bright, probably the mature T cells, and one CD3-dull population that could be a precursor of the dull $CD3^+4^+8^+$ cortical thymocytes as hypothesized by Ceredig *et al.*[19] (Fig. 3*f*). CD3 expression was significantly higher on the mature $CD4^+8^-$ cell population than on the $CD4^-8^+$ cell population (median fluorescence of $CD4^-8^+ = 92.4$ mV; median fluorescence of $CD4^+8^- = 170.7$ mV), suggesting that different T-cell subsets express different levels of TCR/CD3 complex.

In summary, the data presented here and in the accompanying study[11] have shown that the CD3-ε molecule is detectable on a subpopulation of TCRγδ⁺, $CD4^-8^-$ thymocytes early in fetal development and in adult thymi. The function of these cells and the ligand for the TCRγδ/CD3 receptor remains unclear, but we have shown that anti-CD3 mAb can activate these cells to proliferate, secrete lymphokines (data not shown) and develop cytolytic activity in culture.

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Characterization of an expressed CD3-associated Ti γ-chain reveals C_γ domain polymorphism

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The majority of human T cells express an antigen receptor consisting of a disulphide-linked heterodimer (Ti) of relative molecular mass 80,000–90,000 (M_r 80–90K) which is noncovalently associated with a set of at least three proteins of M_r 20–28K termed CD3 (Leu4, T3)[1–3]. Whereas both chains of Ti, an acidic α-chain of M_r 48–54K and a more basic β-chain of M_r 40–44K, contain variable and constant region domains, the component peptides of CD3 are invariant[4–8]. Several laboratories have more recently reported the expression of CD3 in association with a novel protein[9–12]. On the surface of long-term T-cell lines and one thymocyte clone this novel structure consists of a 40K protein noncovalently linked to a 55 or 62K protein[9,10] identified as the protein product of the Ti γ-chain gene, a T-cell specific gene which like the Ti α- and Ti β-chain genes undergoes rearrangement of variable (*V*) and joining (*J*) region gene segments[13–16]. On the human T-cell leukaemic line PEER we have detected only a single 55K glycoprotein associated with CD3[11]. We here demonstrate that an anti-Ti γ-peptide antiserum reacts with the 55K CD3-associated protein on PEER. Most previously described human Ti γ-chain complementary DNA clones encode the products of non-functional rearrangements[17,18]. One of the Ti γ cDNAs isolated from PEER, however, represents a functional rearrangement reported for the first time in a cell which expresses a Ti γ-chain protein product on the cell surface. Interestingly, a 48-base-pair (bp) sequence in the constant (*C*) region domain of this functional Ti γ-chain cDNA is triplicated in PEER and duplicated in other cDNAs isolated from PEER and other cell lines.

In previous studies, a single non-disulphide linked 55K protein was co-immunoprecipitated with CD3 isolated from iodinated cell-surface proteins of PEER[11]. This suggested that the CD3 antigen was not associated with a Ti α/β heterodimer on PEER. To confirm this, we used the monoclonal antibody WT31, which reacts with nonpolymorphic determinants on Ti, and found that PEER failed to react with this antibody by immunoprecipitation (Fig. 1*a* and *b*) or by immunofluorescence[11]. In contrast, WT31 could be used to immunoprecipitate the typical Ti α/β heterodimer expressed on the CD3-bearing leukaemic line, HPB-ALL.

The CD3-associated 55K protein, which was noncovalently linked to a 40K protein on the surface of polyclonal T-cell lines, reacted with antisera generated against peptides derived from the predicted primary sequence of the Ti γ-chain[9,10]. Only one 55K chain appeared to be associated with CD3 on PEER, so it was of interest to determine whether this also represented a Ti γ-chain gene product. We found that one of these previously described anti-Ti-γ-peptide heterosera, generated against a deduced constant region sequence, was reactive with the 55K protein on PEER (Fig. 1*c*). This finding supports the notion that this 55K protein is a product of the Ti γ-chain gene.

Ti β-chain expression precedes Ti α-chain expression but may follow Ti γ-chain expression during thymocyte ontogeny[19–21], so that the formation of Ti γ/β-chain heterodimers has been proposed[19,22]. PEER expresses both Ti β- and Ti γ-chain tran-

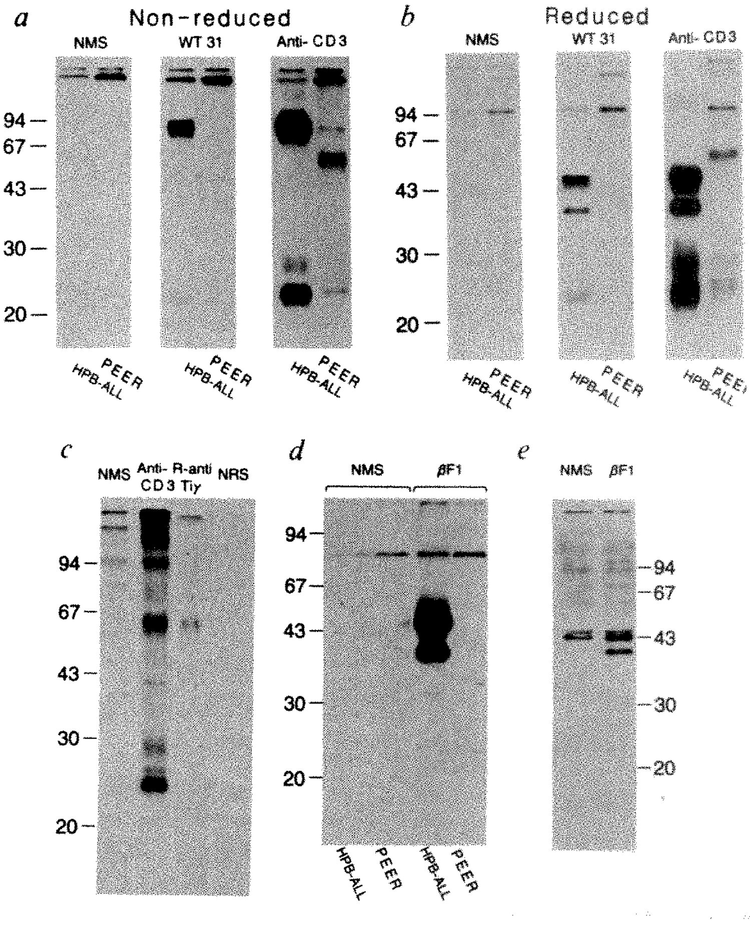


Fig. 1 Characterization of the 55K CD3-associated glycoprotein expressed on PEER. *a*, *b*, WT31 fails to react with the 55K CD3-associated protein. Immunoprecipitation of cell surface radio-iodinated proteins solubilized from HPB-ALL or PEER in 5 mM CHAPS was performed with the indicated preformed immune complexes prepared with rabbit anti-mouse IgG and either normal mouse serum (NMS), WT31 ascites, or anti-CD3 (anti-Leu4) as described[7,8]. Electrophoresis of isolated precipitates was performed under non-reducing (*a*) or reducing (*b*) conditions in 11% sodium dodecyl sulphate (SDS) polyacrylamide gels. *c*, The 55K protein is recognized by an anti-Ti γ peptide serum. An anti-CD3 precipitate prepared from radio-iodinated PEER cells was denatured in 1% SDS containing 2 mM dithiothreitol and heated at 56 °C for 30 min. The sample was renatured with 4 vols of 1.5% Nonidet P40 (NP40), 20 mM iodoacetamide, in Tris-buffered saline, *p*H 7.4. Immunoprecipitation with normal rabbit serum (NRS) or rabbit anti-Ti-γ-peptide (R-anti-Ti-γ) was performed according to the methods of Brenner *et al.*[9]. The rabbit anti-Ti-γ-peptide serum was generated against a synthetic peptide of sequences 117–136 of ref. 18. Immunoprecipitates were analysed as above on 11% gels. *d*, *e*, The β-chain protein is not detected on the surface of PEER but is present intracellularly. Immunoprecipitates prepared from HPB-ALL and PEER cells which were radio-iodinated and lysed in 1% NP40 were isolated with NMS of βF1 monoclonal antibody and analysed as above. In *e*, PEER cell lysates were prepared from cells cultured in ^{35}S-methionine and ^{35}S-cysteine in 1% NP40 as described[8]. Immmunoprecipitates isolated with either preformed complexes containing NMS or βF1 were analysed under reducing conditions in 11% SDS-polyacrylamide gels. Relative mobilities of standards with the indicated molecular masses are shown in the margins.

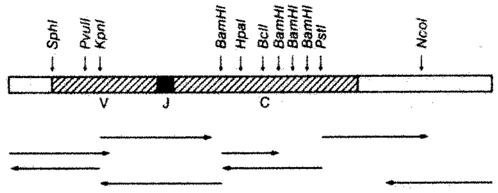
Fig. 2 Nucleotide and predicted amino-acid sequence at the 3′ end of the variable regions of the cDNA clones isolated from PEER. In-frame *V–J* rearrangement, with four additional junctional nucleotides (marked by asterisks) is found in $P_\gamma1$, in which the $V_\gamma8$ gene, a member of the $V_\gamma I$ variable region subgroup, is rearranged to $J_\gamma2$. The non-productive rearrangement on the other chromosome is represented by $P_\gamma12$, in which the $V_\gamma9$ gene, a member of the $V_\gamma II$ subgroup, is rearranged to $J_\gamma2$; there are eight nucleotides of unknown origin at the junction, and there is a deletion of several nucleotides from the *J* segment. The $P_\gamma11$ cDNA clone represents a 2.2-kilobase (kb) transcript of an unrearranged and previously undecribed pseudogene in the $V_\gamma I$ subgroup; the consensus heptamer and nanomer recombination signals, separated by 23 nucleotides, are underlined. *V* regions designated as in ref. 16.

```
                                         +100                                         +110
    Tyr Tyr Cys Ala Thr Trp Lys Asp Tyr  Tyr Lys Lys Leu Phe Gly Ser Gly Thr Thr Leu
5'  TAT TAC TGT GCC ACC TGG AAG GAT TAT  TAT AAG AAA CTC TTT GGC AGT GGA ACA ACA CTT  3'      Pγ1
    <-------- Vγ8 -------->|*** *|<------------------ Jγ2 ------------------------>

    Tyr Tyr Cys Ala Phe Arg Pro  —   Lys Lys Leu Phe Gly Ser Gly Thr Thr Leu
5'  TAC TAC TGT GCC TTC CGG CCC  G   AAG AAA CTC TTT GGC AGT GGA ACA ACA CTT  3'              Pγ12
    |<----- Vγ9 ----->|* *** ***  *|<--------------- Jγ2 ---------------->

    Tyr Tyr Cys Ala Thr Trp
5'  TAT TAC TGT GCC ACC TGG  GGACAGGCACAGTGTTCACACCTGCCCTTACACCACACTGAAAATCTTGGCCTTG  3'     Pγ11
    <------ VγI-family ----->|
```

Fig. 3 Nucleotide sequence of the $P_\gamma1$ cDNA clone and amino-acid sequence of the translated Ti γ-chain protein. *a*, Restriction map of the 1.6-kb $P_\gamma1$ cDNA clone; arrows, M13 inserts sequenced by the dideoxy chain termination procedure[28]. *b*, Sequence of the cDNA and the predicted protein. Numbers above the amino-acid sequence, residue positions in the mature protein; numbers on the right, nucleotide positions. Extracellular cysteines in the variable (*V*) and constant (*C*) regions are marked by stars. The leader sequence (L), *V* domain, *J* segment, and *C* region, which includes the transmembrane (TM) and cytoplasmic (CYT) domains, are indicated by horizontal arrows; *, four nucleotides of uncertain origins at the *V–J* junction. The five potential sites for *N*-linked glycosylation (Asn-X-Thr or Asn-X-Ser) are boxed. The polyadenylation signal, AATAAA, is underlined.

Methods. A cDNA library was prepared in the vector λgt10 using polyadenylated RNA from the T-cell leukaemic line, PEER. The procedure was as described[29], except that size-selected cDNA larger than 1.0 kb was inserted into the *Eco*RI site of λgt10. The library was screened with a full-length γ cDNA clone, $F_\gamma7$, isolated from the T-cell leukaemia FRO2.2[29] (D. Littman, unpublished). Hybridization was performed at 42 °C in 50% formamide, 5 × SSCPE, 10% dextran sulphate, 1 × Denhardt's, and 100 μg ml⁻¹ denatured salmon sperm DNA; the filters were washed in 2 × SSC, 0.1% SDS at 68 °C. Approximately 0.05% of plaques hybridized with the probe $F_\gamma7$.

a



b

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GAATCAGGAAGACCAGCTCCTCCTACTGTCTTCTGTGTTACGGGATCAGCGTTCCTTGTTGAGTGGGACCTGAGTTTTGAGAGGGTCTTCTGCTCCTCTTGGTCTGGTCCCTTACTTCCA  120

                    -20                                     -10                                     -1  +1
                    Met Leu Leu Ala Leu Ala Leu Leu Leu Ala Phe Leu Pro Pro Ala Ser Gln Lys Ser Ser Asn Leu Glu Gly Arg
AGAGCCCCAGAGAGGAAGGC ATG CTG TTG GCT CTA GCT CTG CTT CTA GCT TTC CTG CCT CCT GCC AGT CAG AAA TCT TCC AAC TTG GAA GGG AGA  215
                    |<------------------------------------ L ------------------------------------>|<------ V ------>
                +10                             +20  *                              +30
Thr Lys Ser Val Thr Arg Pro Thr Gly Ser Ser Ala Val Ile Thr Cys Asp Leu Pro Val Glu Asn Ala Val Tyr Thr His Trp Tyr Leu
ACA AAG TCA GTC ACC AGG CCA ACT GGG TCA TCA GCT GTA ATC ACT TGT GAT CTT CCT GTA GAA AAT GCC GTC TAC ACC CAC TGG TAC CTA  305
------ V ------ -- -- --
                +40                             +50                                 +60
His Gln Glu Gly Lys Ala Pro Gln Arg Leu Leu Tyr Tyr Asp Ser Tyr Asn Ser Arg Val Val Leu Glu Ser Gly Ile Ser Arg Glu Lys
CAC CAG GAG GGG AAG GCC CCA CAG CGT CTT CTG TAC TAT GAC TCC TAC AAC TCC AGG GTT GTG TTG GAA TCA GGA ATC AGT CGA GAA AAG  395

                +70                             +80                                 +90         *
Tyr His Thr Tyr Ala Ser Thr Gly Lys Ser Leu Lys Phe Ile Leu Glu Asn Leu Ile Glu Arg Asp Ser Gly Val Tyr Tyr Cys Ala Thr
TAT CAT ACT TAT GCA AGC ACA GGG AAG AGC CTT AAA TTT ATA CTG GAA AAT CTA ATT GAA CGT GAC TCT GGG GTC TAT TAC TGT GCC ACC  485
                                                                                   -- -- -- ----------- V -----------
                +100                            +110                                +120
Trp Lys Asp Tyr Tyr Lys Lys Leu Phe Gly Ser Gly Thr Thr Leu Val Val Thr Asp Lys Gln Leu Asp Ala Asp Val Ser Pro Lys Pro
TGG AAG GAT TAT TAT AAG AAA CTC TTT GGC AGT GGA ACA ACA CTT GTT GTC ACA GAT AAA CAA CTT GAT GCA GAT GTT TCC CCC AAG CCC  575
-->|*** *|<----------------------- J ------------------------>|<------------------ C ------------------ -- -- --
                +130                            +140                    *           +150
Thr Ile Phe Leu Pro Ser Ile Ala Glu Thr Lys Leu Gln Lys Ala Gly Thr Tyr Leu Cys Leu Leu Glu Lys Phe Phe Pro Asp Ile Ile
ACT ATT TTT CTT CCT TCG ATT GCT GAA ACA AAA CTC CAG AAG GCT GGA ACA TAT CTT TGT CTT CTT GAG AAA TTT TTC CCA GAT ATT ATT  665

                +160                            +170                                +180
Lys Ile His Trp Gln Glu Lys Lys Ser Asn Thr Ile Leu Gly Ser Gln Glu Gly Asn Thr Met Lys Thr Asn Asp Thr Tyr Met Lys Phe
AAG ATA CAT TGG CAA GAA AAG AAG AGC AAC ACG ATT CTG GGA TCC CAG GAG GGG AAC ACC ATG AAG ACT AAC GAC ACA TAC ATG AAA TTT  755

                +190                            +200  *                             +210
Ser Trp Leu Thr Val Pro Glu Glu Ser Leu Asp Lys Glu His Arg Cys Ile Val Arg His Glu Asn Asn Lys Asn Gly Ile Asp Gln Glu
AGC TGG TTA ACG GTG CCA GAA GAG TCA CTG GAC AAA GAA CAC AGA TGT ATC GTC AGA CAT GAG AAT AAT AAA AAC GGA ATT GAT CAA GAA  845

                +220                            +230                                +240
Ile Ile Phe Pro Pro Ile Lys Thr Asp Val Thr Thr Val Asp Pro Lys Asp Ser Tyr Ser Lys Asp Ala Asn Asp Val Thr Thr Val Asp
ATT ATC TTT CCT CCA ATA AAG ACA GAT GTC ACC ACA GTG GAT CCC AAA GAC AGT TAT TCA AAA GAT GCA AAT GAT GTC ACC ACA GTG GAT  935

                +250                            +260                                +270
Pro Lys Tyr Asn Tyr Ser Lys Asp Ala Asn Asp Val Ile Thr Met Asp Pro Lys Asp Asn Trp Ser Lys Asp Ala Asn Asp Thr Leu Leu
CCC AAA TAC AAT TAT TCA AAG GAT GCA AAT GAT GTC ATC ACA ATG GAT CCC AAA GAC AAT TGG TCA AAA GAT GCA AAT GAT ACA CTA CTG 1025

                +280                            +290                                +300
Leu Gln Leu Thr Asn Thr Ser Ala Tyr Tyr Met Tyr Leu Leu Leu Leu Leu Lys Ser Val Val Tyr Phe Ala Ile Ile Thr Cys Cys Leu
CTG CAG CTC ACA AAC ACC TCT GCA TAT TAC ATG TAC CTC CTC CTG CTC CTC AAG AGT GTG GTC TAT TTT GCC ATC ATC ACC TGC TGT CTG 1115
                            |<------------------------------ TM ----------------------------------------------------
                +310                    +318
Leu Gly Arg Thr Ala Phe Cys Cys Asn Gly Glu Lys Ser ---
CTT GGA AGA ACG GCT TTC TGC TGC AAT GGA GAG AAA TCA TAA CAGACGGTGGCACAAGGAGGCCATCTTTTCCTCATCGGTTATTGTCCCTAGAAGCGTCTTCTGA 1221
------>|<------------ CYT ------------>|
GGATCTAGTTGGGCTTTCTTTCTGGGTTTGGGCCATTTCAGTTCTCATGTGTGTACTATTCTATCATTATTGTATAATGGTTTTCAAACCAGTGGGCACACAGAGAACCTCAGTCTGTAA 1341

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TCCCTCTCTTAGTGTTCTTTAATCAGATAACTGCCTGGAAGCCTTTCATTTTACACGCCCTGAAGCAGTCTTCTTTGCTAGTTGAATTATGTGGTGTGTTTTTTCCGTAATAAGCAAAATA 1581

AATTTAAAAAAAAAAAAAA  1600
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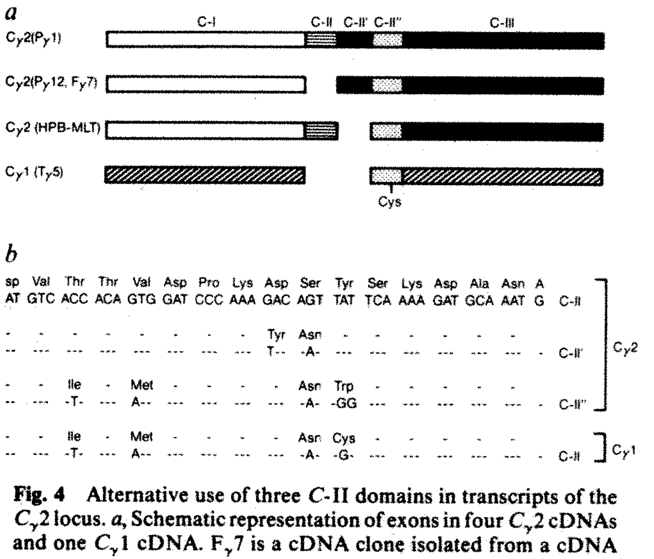

Fig. 4 Alternative use of three *C*-II domains in transcripts of the $C_\gamma 2$ locus. *a*, Schematic representation of exons in four $C_\gamma 2$ cDNAs and one $C_\gamma 1$ cDNA. $F_\gamma 7$ is a cDNA clone isolated from a cDNA library of the FRO2.2 leukaemia (P.-G. Pelicci *et al.*, in preparation): $T_\gamma 5$ is a cDNA clone isolated from a human peripheral T-cell library (D.L., unpublished result). The *C*-II domain of the $C_\gamma 1$ locus differs from the *C*-II domain of $C_\gamma 2$ at a single nucleotide, resulting in replacement of the tryptophan with a cysteine. *b*, Sequence of the three *C*-II domains from the four $C_\gamma 2$ cDNAs and the homologous sequence of $C_\gamma 1$.

scripts, but fails to express Ti α-chain messenger RNA. This finding suggested that a Ti γ/β heterodimer might be expressed on PEER. To assess this, the monoclonal antibody (mAb) βF1, which reacts specifically with all Ti β-chains[9], was used in immunoprecipitation studies with PEER.

Although we failed to detect Ti β chains in lysates prepared from cell-surface iodinated PEER cells, a 41K Ti β-chain protein could be detected in lysates prepared from metabolically labelled cells (Fig. 1*d* and *e*). This indicates that Ti β-chains in PEER are not expressed on the cell surface. This excludes the possibility of Ti β/γ heterodimers on PEER.

All human Ti γ-chain cDNAs isolated to date have resulted from non-productive *V–J* rearrangements[17,18]. Therefore, isolation of an in-frame functional Ti γ-chain cDNA from PEER would further indicate that the 55K protein on PEER is a Ti γ-gene product. As the 55K protein on PEER is not expressed in the anticipated heterodimer configuration, examination of the primary Ti γ-chain sequence should be informative. A cDNA library of PEER was constructed in the λgt10 phage vector. Of six Ti γ-chain cDNA clones isolated and sequenced, clones corresponding to three distinct transcripts were identified. Two clones, represented by $P_\gamma 1$, contain full-length sequences with in-frame rearrangements (Figs 2 and 3). Three other identical clones, represented by $P_\gamma 12$, are the result of an out-of-frame joining event (Fig. 2). A third clone, $P_\gamma 11$, corresponds to the product of a previously undescribed unrearranged *V* region pseudogene, a member of the V_γI subgroup (Fig. 2). This product may be analogous to upstream *V* region transcripts observed in the immunoglobulin (Ig) heavy-chain locus which can undergo secondary rearrangements resulting in *V* region replacements[23,24]. As in V_H regions, a consensus heptamer sequence (TACTGTG) is present at the 3′ end of all human V_γ segments. As there is differential usage of V_γ genes during murine thymocyte development, it has been proposed that such upstream V_γ genes may be involved in secondary rearrangements[25]. The finding of a germline V_γ region transcript in PEER suggests that the Ti γ locus remains accessible for further rearrangements, and is consistent with this model.

Examination of the sequence of $P_\gamma 1$ reveals several interesting features (Fig. 3). (1) This sequence is the result of in-frame joining of the $V_\gamma 8$ gene segment (a member of the $V_\gamma I$ subgroup) to $J_\gamma 2$[16]. Four additional nucleotides are found at the *V–J* junction; these are likely to be *N* nucleotides but may represent a previously undetected *D* region. (2) The constant region of this clone is nearly identical to the recently isolated $C_\gamma 2$ cDNA[18] and, in contrast to products of the $C_\gamma 1$ locus (see below), lacks cysteines external to the plasma membrane other than those in the immunoglobulin-like domain (Fig. 4). This is of particular interest as the 55K CD3-associated chain does not have a disulphide-linked partner chain, typically seen in Ti α/β heterodimers. (3) The sequence contains five potential sites for *N*-linked glycosylation, compatible with the reported high degree of endoglycosidase F sensitivity of the 55K CD3-associated chain[11]. (4) Of considerable interest is a 48-base-pair (bp) triplication within the *C* region. Duplication of this 48-bp segment in the $C_\gamma 2$ of HPB-MLT has been described[18], and we confirmed the presence of this triplication in PEER by sequencing a second independent cDNA clone containing $V_\gamma 8$ and found its *C* region sequence to be identical to $P_\gamma 1$.

A close examination of the 48-bp repeat of $P_\gamma 1$ reveals that these 48-bp segments are not exact repeats but contain 2- or 5-bp differences, allowing discrimination between these repeated sequences (Fig. 4*b*). The *C* region found in the non-functionally rearranged Ti γ cDNA from PEER, $P_\gamma 12$, also contains a $C_\gamma 2$-like sequence with a duplication of 48-bp segments, but one segment is distinct from either of those described by Dialynas *et al.*[18]. We have isolated another Ti γ cDNA from the cell line FRO2.2 which also contains a 48-bp duplication identical to that of $P_\gamma 12$. All the duplicated 48-bp segments in these cDNA clones are represented in the triplicated segments seen in PEER (Fig. 4*a*). Thus, three distinct and very similar 48-bp sequences are observed and are variably used in the same or different cells as indicated in Fig. 4*a*.

The third segment in the triplication in $P_\gamma 1$ is very similar to a single 48-bp segment contained within the constant region of a cDNA corresponding to the $C_\gamma 1$ locus (Fig. 4); the only difference is a substitution of cysteine for tryptophan in the homologous segment of the $C_\gamma 1$ gene. No other cysteines, excluding the two in the immunoglobulin-like region, are found external to the transmembrane domain in $C_\gamma 1$. In addition to the differences illustrated in Fig. 4, there are only five other amino-acid differences between products of the $C_\gamma 1$ and $C_\gamma 2$ loci (P. G. Pelicci *et al.*, in preparation).

The mechanism by which the distinct 48-bp repeats are variably expressed in $C_\gamma 2$ is not readily apparent, but several explanations can be offered. Preliminary studies indicate that all three exist in the $C_\gamma 2$ gene as separate exons which may have arisen recently by gene duplication (P. G. Pelicci *et al.*, in preparation). The variable expression of these 48-bp segments is likely to result from distinct alleles of the $C_\gamma 2$ locus. Alternatively, although less likely, these segments may be variably used by alternative splicing or they may be present in other C_γ genes which remain to be detected. No comparable duplications have been described in the mouse, but these segments exhibit limited homology to a 69-bp segment in the recently-described murine $C_\gamma 4$ gene, which is absent from the murine $C_\gamma 1$–3 genes[26]. The explanation of the origin of these repeats awaits a more detailed analysis of the human Ti γ genomic organization.

The functional significance of these 48-bp repeats is unclear. A consequence of their presence in $C_\gamma 2$ is the absence of a cysteine residue which is found within the highly homologous $C_\gamma 1$ protein domain. This accounts for the failure of the Ti γ glycoprotein to form a disulphide-linked dimer on PEER and presumably other cells using $C_\gamma 2$. Whether the CD3-associated Ti γ-chain on PEER is a single chain or a non-disulphide-linked homodimer or heterodimer remains unclear. In view of the differences between $C_\gamma 1$ and $C_\gamma 2$ and of the variable expression of the 48-bp segments in $C_\gamma 2$ sequences, it may be anticipated that different protein products of the Ti γ-chain locus will be observed. The non-disulphide-linked CD3 protein observed on PEER and the T-cell lines and the one thymocyte clone reported[9-11] are likely to represent the expression of products of the $C_\gamma 2$ locus. Recent studies demonstrating CD3$^+$, WT31$^-$ cells on which a disulphide linked 80-90K dimer is associated with CD3 may represent the expression of protein products of the $C_\gamma 1$ locus[12,27]. Further differences in $C_\gamma 2$ gene products are likely to be observed, as there are three potential *N*-linked glycosylation sites in the triplicated 48-bp segments; variable use of these 48-bp segments may result in substantial changes in molecular mass of the expressed Ti γ-chain protein. The ability of such different products of the C_γ-gene loci to function as receptors or as signal-transducing molecules may also differ.

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Absence of both cytochrome b_{-245} subunits from neutrophils in X-linked chronic granulomatous disease

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Phagocytosis by neutrophils and other 'professional' phagocytic cells is accompanied by a microbicidal burst of non-mitochondrial respiration[1]. Cytochrome b_{-245} is the only clearly defined component of this oxidase system and its absence[2] provides the molecular basis of X-linked chronic granulomatous disease (CGD), in which a profound predisposition to infection results from complete failure of this respiratory burst[3]. Purification of the cytochrome has proved difficult, with uniform disagreement regarding the identity of its apoprotein, descriptions of its relative molecular mass (M_r) on SDS-polyacrylamide gel electrophoresis (PAGE) ranging from 10,000 to 127,000 (10-127K)[4-8]. I report here that it has two subunits, a 23K protein and the previously described 76-92K glycoprotein[9]. These subunits are closely linked and remain associated with the haem of the cytochrome through affinity and gel filtration chromatography and sucrose gradient centrifugation, and exhibit a similar distribution in a *p*H gradient. Neither protein was detected in the cells of five patients with X-linked CGD whereas both were present in two with the form of this disease with autosomal recessive inheritance.

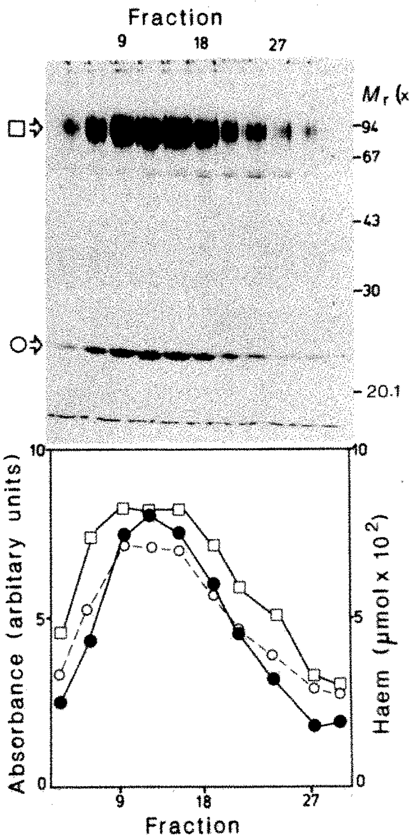

Fig. 1 SDS-PAGE of fractions collected during the elution of highly purified cytochrome b_{-245} of neutrophils from a heparin-agarose affinity column. Two main bands of protein with apparent M_rs on gels of 76-92K (□) and 23K (O) co-purify with the haemoprotein (●) of the cytochrome (below).

Methods. Neutrophils (400 ml packed cells) were fractionated on discontinuous sucrose gradients[22] and the membranes and specific granules, with a density of less than 1.21, were pelleted and extracted into buffer A[4] containing EDTA (1 mM *p*H 7.4), phenylmethylsulphonyl fluoride (1 mM), Trasylol (100,000 iu ml^{-1}), tosyl-L-lysine chloromethyl ketone (TLCK, 27 µM) as described[4]. After passing the extract sequentially through columns of CM and DEAE fast-flow Sepharoses (30 ml, Pharmacia Fine Chemicals) and *N*-amino-octyl-Sepharose (15 ml, Sigma) the cytochrome was bound to a column of heparin agarose (10 ml, Sigma) from which it was eluted with a gradient of NaCl (30 ml, 0.1-0.4 M) and collected in 1 ml fractions. An aliquot (100 µl) from every third fraction was run on a 10% SDS-polyacylamide gel with the Laemmli system of buffers[23] and stained with Coomassie blue. The peak specific activity (fraction 15) was 29.6 nmol haem per mg protein. The gel was scanned with a Chromoscan III scanner (Joyce Loebl). Comparison by regression analysis of the height of the peak of absorbance by the 76-92K band with that of the 23K band in the same track gave an *r* value of 9.69. The value was 9.40 when compared with the haem signal at 558 nm in the corresponding fraction.

The molecular basis of the respiratory burst of neutrophils, monocytes, macrophages and eosinophils that is important for the killing[10] and digestion[11] of microbes by phagocytic cells has posed an interesting problem[12]. Attention was focused on cytochrome b_{-245} when it was identified as a component of this oxidase system and its absence defined as the molecular basis of the X-linked subtype of CGD[2].

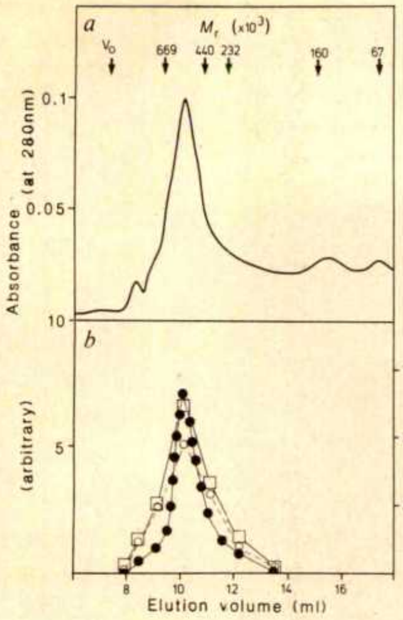

Fig. 2 Gel filtration chromatography of cytochrome b_{-245} in which the 23K (○) and 76-92K (□) proteins co-eluted with the haem of the cytochrome (●) with an apparent M_r of ~500-600K. **Methods.** Highly purified cytochrome *b* (6.8 nmol in 200 μl) was chromatographed in buffer A containing 1% Triton N101 on a FPLC Superose 12 column (1×30 cm, Pharmacia) at a flow rate of 0.1 ml min^{-1}. The peak of absorption at 280 nm, at a retention volume of 10.18 ml, coincided with the peaks of elution of the haem of the cytochrome, and of the two protein bands as measured by quantitative scanning of SDS-PAGE electrophoretograms. The size markers were blue dextran (void volume), thyroglobulin (669K), ferritin (440K), catalase (232K), immunoglobulin G (160K) and albumin (67K) which were chromatographed in buffer A in the absence of dithiothreitol.

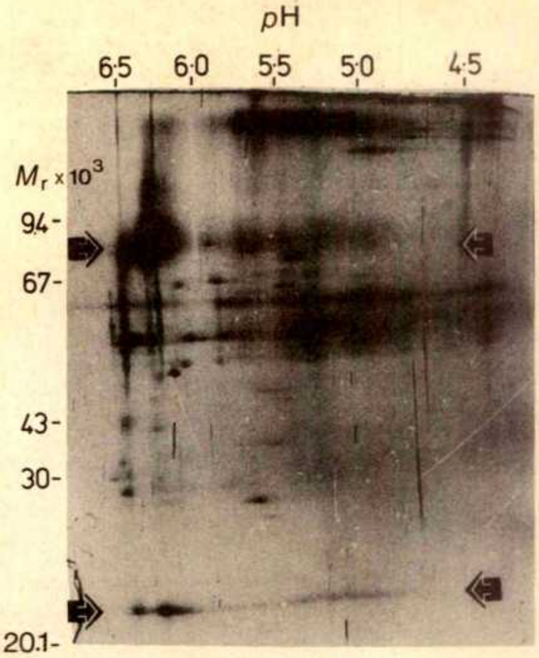

Fig. 3 Two-dimensional gel electrophoresis of cytochrome b_{-245}. The 23K and 76-92K proteins (arrows) showed a similar pattern of distribution in the *p*H gradient.
Methods. Separation in the first dimension was by the method of O'Farrell[24]. An aliquot (200 μl) of fraction 15 from the preparation described in Fig. 1 was dialysed at 25 °C for 2 h against lysis buffer (9.5 M urea, 2% Nonidet P40, 5% 2-mercaptoethanol), applied to the acid end of the rod containing 2% Pharmalyte 3-10 (Pharmacia) and focused for 1,600 V h. The rod was then soaked in sample buffer[23] for 15 min, electrophoresed into the second dimension of 10% acrylamide, and silver stained[13]. Only the region of the gel in which the proteins were observed is shown.

Improvements in the purification of the cytochrome, by the use of additional protease inhibitors and more rapid separation techniques, enabled us to isolate a protein of M_r 23K (23.49±8, $n=7$) in addition to the customary broad protein band at 76-92K (s.ds 8.4 and 5.3 respectively, $n=7$, on 7.5-15% SDS-PAGE gels). Further studies were undertaken to examine the relationship of these two proteins to the cytochrome.

The association of the two proteins with each other and with the haem spectrum of cytochrome b_{-245} was determined when the latter was purified on heparin agarose (Fig. 1), and then subjected to gel filtration chromatography (Fig. 2). All three demonstrated an essentially identical elution profile from both columns. Assuming similar binding of Coomassie blue, the proteins appeared to be associated in roughly equimolar amounts. Essentially similar results were observed after the cytochrome was centrifuged into a linear gradient of sucrose (20-50%, containing 1% Triton N101, 130,000*g* for 20 h in a Sorvall TV-850 vertical rotor) in which it banded with a modal density of ~1.12 (results not shown). Isoelectric focusing of the purified cytochrome in the presence of 8 M urea resulted in the same pattern of distribution of both proteins (Fig. 3). Although small amounts of both proteins were widely distributed in the first dimension, a large proportion of both focused with a *p*I of ~6.0-6.5.

The demonstration of the absence or abnormality of one or both of these proteins in X-linked CGD would provide strong additional evidence for their association with the cytochrome, the haem spectrum of which is missing in this condition[2]. I therefore modified our described method[4] to allow the purification of this cytochrome from the 10^8 cells readily obtainable from peripheral blood samples (Fig. 4 legend). This results in a relatively high recovery of the cytochrome (44.5±19%, $n=4$) to a degree of purity (11.7±3.2 nmol haem per mg protein, $n=4$) that compares well with the 5.3, 9-13, 16 and 20 nmol mg^{-1} reported for large-scale purifications (refs 5, 8, 4 and 6 respectively). After this purification the 23K and 76-92K proteins could be easily identified on SDS-PAGE electrophoretograms and as little as 10 pmol of the cytochrome (the amount present in 10^6 neutrophils) was clearly visible in a track after silver staining[13] the gel (Fig. 4).

The same procedure was then adopted with neutrophils from five male patients with X-linked CGD all of whose cells lacked spectral evidence of the cytochrome (patients nos 4, 14 and 19 in ref. 2, and two newly diagnosed boys with heterozygote carrier mothers). For comparison, two patients with an autosomal recessive pattern of inheritance of the disease, whose cells contained the cytochrome, a 20-year-old girl (patient no. 23, ref. 2) and a 41-year-old man, were also studied. In contrast to the presence of both these proteins in extracts from all ten normal individuals and the two patients with autosomal recessive CGD, both the 76-92K and the 23K proteins were undetectable in all five of the X-linked CGD patients (Fig. 4), clearly associating them with each other and with the cytochrome.

The purification of the complex of these two proteins almost certainly depends upon the binding of one, probably the 76-92K subunit[4], to the heparin agarose. Thus both proteins might appear to be absent from cells lacking only that subunit carrying the requirements for purification. To examine this possibility, antibodies were raised to the 23K protein and used to probe Western blots of SDS-PAGE electrophoretograms of whole membranes. The antigen was detected in membranes from seven normal subjects and the female autosomal recessive patient, but

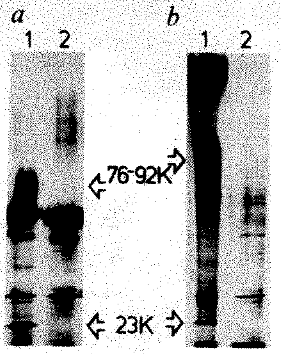

Fig. 4 *a*, *b*, Two different comparisons of the pattern of proteins in extracts purified from two normal subjects (tracks in *a* and *b*) and two patients with X-linked CGD (tracks 2). The 76-92K and 23K protein bands (arrows) observed in extracts from normal cells are absent from those of the patients.

Methods. Neutrophils were purified from 100 ml venous blood[25] and pelleted at 600*g* at 20 °C for 5 min. Diisopropyl fluorophosphate (5 μg) was added to the pellet and the cells were then resuspended in buffer A (see Fig. 1) without detergent and centrifuged at 80,000*g* at 4 °C for 15 min. The cell pellet was extracted twice with sodium cholate (1% w/w) in buffer A and then washed in buffer A without detergent. The cytochrome was finally extracted twice in buffer A containing Triton N101 (1% w/w). Each time, the pellet was homogenized in the centrifuge tube in 8 ml of the appropriate buffer for 10 s at 24,000 r.p.m. with a Polytron homogenizer and centrifuged as described above. The Triton N101 extract was then passed (5 ml h^{-1}) successively through: CM Sepharose; DEAE Sepharose; *N*-amino-octyl Sepharose; (1.5 ml bed volume of each resin) and then bound to heparin agarose (400 μl bed volume). The extract was followed with buffer A containing Triton N101 (10 ml at 5 ml h^{-1}) after which the heparin agarose column was disconnected, washed with 5 ml of buffer A containing Triton N101 and NaCl (0.1 M) and the cytochrome was then eluted with the same buffer containing 0.7 M NaCl. The first 240 μl were discarded and the eluate was collected in 150 μl fractions, with the peak recovery of the cytochrome in the second and third fractions. The cytochrome was quantified from the reduced minus oxidized difference spectrum taking $\Delta E_{559-540}$ = 21.6 cm^{-1} mM^{-1} for the α band[18]. Protein was measured with the Pierce BCA reagent with bovine serum albumin as standard. Electrophoresis of all fractions was in 0.75-mm thick 5-15% slab gels which were silver stained[13]. The peak fraction of elution of the cytochrome from normal cells (fraction 3) is shown analysed in tracks 1 (21 and 16 pmol haem in tracks 1*a* and 1*b* respectively) and the corresponding fractions from the patients in tracks 2. The 23K and 76-92K proteins were not observed in any fractions from any of the five X-linked CGD patients.

not in those of all five X-linked patients studied (Fig. 5). Unfortunately these studies could not be extended to the 76-92K protein as we have been unable to raise suitable antisera, and antibodies against the 23K protein do not appear to immunoprecipitate the complex.

The active oxidase is thought to contain a flavoprotein[14,15] in addition to cytochrome b_{-245} but unfortunately the identification of their respective apoproteins has been hampered by the displacement of both the haem and flavin adenine dinucleotide (FAD) by mild denaturation. Hence, one of the subunits could be the apoprotein of the cytochrome and the other of the flavoprotein. It is interesting in this context that the FAD content of neutrophil membranes was determined in three of the five patients with X-linked CGD in this study, in all of whom it was found to be abnormally low[14], although reduced levels of membrane-associated FAD has not been a consistent finding in this condition in the hands of other investigators[16].

I propose that conventional terminology is adopted and the 23K and 76-92K proteins called the α and β subunits respectively, assigning β to the larger subunit as it seems more probable that this is the haem-binding apoprotein: in our earlier purifications of the cytochrome it was seen as a single band[4]. The gel filtration studies indicated an M_r for the cytochrome of ~500-600K. Although the anomalous behaviour of membrane proteins in detergent on gel filtration columns makes accurate predictions of molecular size impossible[17], it would not be surprising if these subunit assemblies were arranged in dimers or tetramers. Although such quaternary structure would be very unusual for a mammalian cytochrome *b*, we know this cytochrome to be atypical, resembling bacterial cytochromes *o*[18] which have between 1 and 4 subunits[19].

Orkin's group recently cloned a gene on the X chromosome[20] that they found to be abnormal in X-linked CGD. It appears to encode a protein that appears different from both subunits

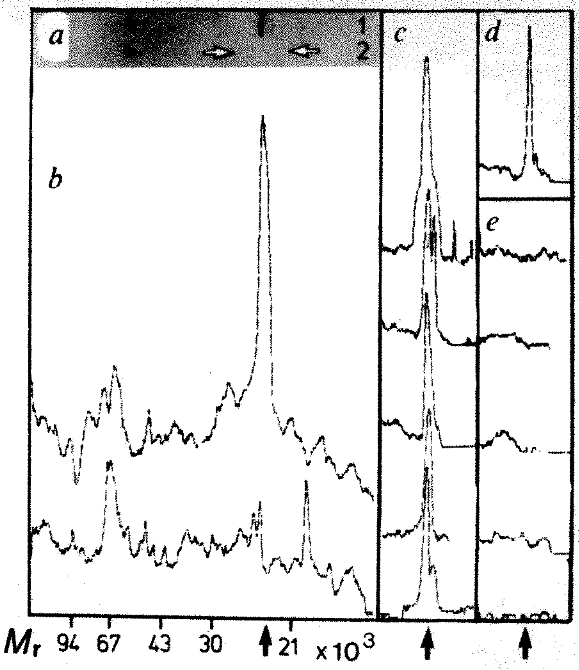

Fig. 5 Immunoblot of neutrophil membranes using antiserum to the 23K protein (*a*) and reflectance scans of blots against normal subjects (*b–e*), confirming the absence of this protein from five patients with X-linked CGD (in *a*, track 2 (horizontal arrows)); in *b*, lower scan, and *e*, but not CGD with an autosomal recessive pattern of inheritance (*d*). A blot (*a*, upper track) and 6 scans from different normal subjects (*b*, upper scan, and *c*) are also shown. Arrows on the horizontal axis mark the position of the 23K protein.

Methods. Purified cytochrome b_{-245} (35 nmol haem, 2 mg protein) was subjected to SDS-PAGE on a 10% slab gel. The band of protein at 23K was electroeluted from the finely-chopped acrylamide in a dialysis sac in Tris/glycine/SDS[23] running buffer at 4 °C at 320 V for 18 h. An aliquot of the eluted protein (50 μg) was inoculated with Freunds complete adjuvant by multiple subcutaneous injections into New Zealand White rabbits (2 kg body weight) followed by four further injections in Freunds incomplete adjuvant at two-weekly intervals. Membrane proteins (*a*, *b*, 40 μg; *c–e*, 20 μg per track) were run on a 12.5% gel and blotted onto nitrocellulose for 3 h at 550 mA in running buffer containing 20% methanol. The blot was washed in Tris/HCl (50 mM, *p*H 7.4) buffered NaCl (200 mM) containing Tween 20 (0.05%) (TBS/Tween 20) for 10 min, blocked with bovine serum albumin (3%) and normal goat serum (10%) in TBS/Tween 20 for 10 min and incubated at room temperature in the antiserum (1 in 25 dilution in blocking buffer) for 2 h, washed in TBS/Tween for 10 min, washed for 10 min in block buffer, incubated with goat anti-rabbit IgG (H & L) horseradish peroxidase conjugate (Biorad, Western Blotting Grade, 1 in 2,000 in block buffer) for 1 h at 25 °C, washed for 5 min in TBS/Tween and developed with 4-chloro-1-naphthol (2.8 mM) and H_2O_2 (4.4 mM) in TBS at 25 °C. Minor coloured bands were sometimes observed at about 60K and 18K in the absence of the first antiserum.

of cytochrome b_{-245}: it has a dissimilar amino-acid composition from the β subunit[9] and its predicted M_r of 54K is more than twice that of the α subunit. It could be another redox component of the oxidase system, although it does not have an obvious haem[20] or nucleotide[21] binding site, or an associated structural protein. If, as they claim, the gene they have cloned is the site of the primary lesion in X-linked CGD, the identification of the gene product in question will be required in order to understand its relationship to the phenotypic absence of the protein subunits of cytochrome b_{-245} described here.

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Linkage of an X-chromosome cleft palate gene

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Many congenital malformations, such as cleft palate and neural tube defects, have a multifactorial origin involving both environmental and genetic factors. Conditions such as these may be exclusively monogenic, polygenic or environmental, but in most cases both genetic and environmental factors are involved[1]. This study describes the sub-chromosomal localization of a single gene defect causing cleft palate and ankyloglossia (tongue-tied) in a large Icelandic family. This defect is a model for the analysis of other neural-crest malformations that show a more complex multifactorial inheritance pattern.

Human secondary cleft palate (CP) occurs during the seventh to ninth weeks of embryological development when the lateral palatine shelves fail to fuse[2]. This malformation shows little variation between populations; the incidence is ~1 in 1,500[3]. CP is more prevalent in females than in males[4], which may be because the palatine processes fuse one week later in females[5], allowing more time for teratogenic agents to play a part in the potential for malformation in females.

The analysis of single gene mutations using restriction fragment length polymorphisms (RFLPs) for linkage studies has been successful in determining the chromosomal location of several common inherited disorders. Linkage to within 5 centimorgans has been detected, both in cases where the affected chromosome is known, as for the sex-linked disorders Duchenne muscular dystrophy[6] and chronic granulomatous disease[7], and when neither the autosome nor the biochemical defect is identified, as for Huntington's disease[8] and cystic fibrosis[9-11]. However, the analysis of polygenic disorders by linkage studies with RFLP markers is at present more complex both theoretically and practically.

One way to learn more about the aetiology of disorders with complex combinations of genetic and environmental factors is to use as a model a family in which the phenotype is due to a single gene defect, but displays the same features as more common multifactorial sporadic cases. Such a model for midline congenital defects has been found in a large Icelandic family

Table 1 X-chromosome probes that show linkage to the CP+A locus: their localization, polymorphism and lod scores at various recombination fractions (θ).

Probe	X-chromosome location	Polymorphism-enzyme	lod at θ value						
			0	0.01	0.05	0.10	0.20	0.30	0.40
L1.28	p11.3	*Taq*I	0.38	0.37	0.36	0.33	0.27	0.19	0.10
p58.1	p11-cen	*Msp*I	0.65	0.65	0.64	0.62	0.52	0.38	0.21
cpX203	p1.1	*Bgl*II	$-\infty$	−0.33	0.22	0.40	0.46	0.37	0.21
pDP34	q13-q21	*Taq*I	3.07	3.02	2.81	2.53	1.92	1.28	0.62
X65H7	q13-q22	*Hin*dIII	$-\infty$	−0.03	0.92	1.06	0.97	0.71	0.38
7b	q13-q22	*Pst*I	$-\infty$	−0.53	0.18	0.37	0.44	0.36	0.21

All likelihoods and lod scores were calculated with the LINKAGE computer program package[20]. Individuals with either cleft palate and/or ankyloglossia were considered to be uniformly affected (that is both phenotypes combined to one 'affection status') and the maximal likelihood estimate for the penetrance (t) of this single gene for 'carrier females' was calculated to be 82%. The lod scores (Z) were calculated according to Ott[21]: $Z(\theta) = \log$ likelihood $(\theta, t) - \log$ likelihood $(1/2, t)$, where $t = 0.82$. The frequency for the CP+A mutant allele was taken as 0.0001. Likelihoods calculated at a range of penetrances, RFLP and CP+A allele frequencies made no significant difference to the quoted lod scores (data available on request). Respectively 3, 6, 4, 8, 6 and 5 meioses contributed most of the linkage data for probes L1.28, p58.1, cpX203, pDP34, X65H7 and 7b (for probe localization see ref. 22).

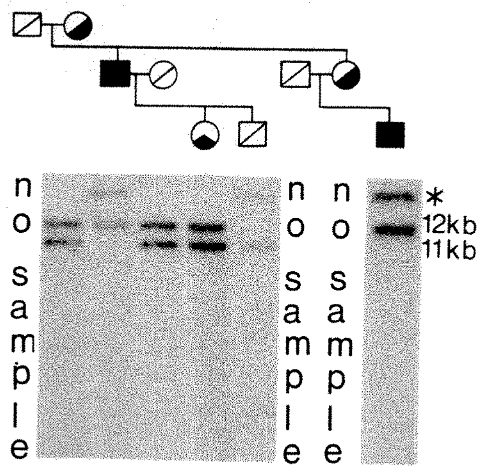

Fig. 1 *Taq*I-digested DNA samples from the pedigree are shown hybridized to the anonymous linked pDP34 marker. Section of the Icelandic family showing the X-linked inheritance of CP and A (⧄ or ⊘, unaffected ♂ and ♀; ■, affected ♂ CP+A; ◑, affected ♀, A alone; ◔, obligatory carrier; *, Y-specific band).

Method. Genomic DNA was prepared from 10 ml of whole blood[23]. DNA ($4\,\mu g$) was digested with the relevant restriction enzyme revealing the polymorphism for each X-chromosome probe. The digested DNA was fractionated by electrophoresis on 1% agarose gels and transferred to Hybond-N membranes (Amersham)[24]. The probes used were labelled to a specific activity of 1×10^9 d.p.m. μg^{-1} by synthesis using random oligonucleotide primers. The filters were washed down to a final salt concentration of 0.1×SSC (SSC is 0.15 M NaCl, 0.015 M sodium citrate) in the presence of 0.1% SDS at 65 °C. Autoradiography was for 24 h at −70 °C with an intensifying screen.

(over 200 members) showing mendelian inheritance of X-linked secondary cleft palate and ankyloglossia ('tongue-tied') (CP+A). Both the large size of this pedigree and the availability of many defined X-chromosome probes has made it possible to localize this defect sub-chromosomally.

A section of the pedigree of 293 individuals in the family studied is shown in Fig. 1; three of us (A.B., A.A. and O.J.) have personally investigated 182 individuals in four living generations, and information on other family members was gained either from written or family records[12]. (Full pedigree data are available from the authors on request.) Blood was collected from 82 individuals for RFLP genotyping, including 9 males with secondary cleft palate and ankyloglossia (CP+A) and 10 females with ankyloglossia alone (A). One female had CP alone and one male had a patent but high-vaulted palate (HVP) characteristic of those affected. For purposes of linkage analysis each family member was designated affected if they had any of the features diagnostic for CP, A or HVP.

Of the 14 matings where a CP+A father has the opportunity to pass a mutant gene to his son, the son is never affected. This suggests that this gene is sex-linked. Occasionally, an unaffected female has affected sons and daughters, and is postulated to be a carrier; the maximal likelihood estimate for the penetrance of the mutation is calculated to be 82% (Table 1).

The analysis of inheritance of RFLPs was carried out using standard techniques (Fig. 1). Linkage at a lod score of 3.07 has been demonstrated between the CP+A locus and the locus *DXYS1* of the anonymous DNA probe pDP34, which maps to Xq13-Xq21[13] (Table 1). Further linkage analysis of the loci defined by an additional 14 informative probes has allowed exclusion of most of the rest of the X chromosome.

Localization of the mutation causing cleft palate in this family is a first step in understanding the genetic component of congenital neural tube and crest defects. This region of the X chromosome contains an X-Y homologous region[14]; the availability of many X-Y homologous probes should permit the fine mapping of the defect. As the limits of genetic mapping in this family are approached the techniques of cosmid walking and jumping and pulsed-field gel electrophoresis[15,16] will allow the gap between the genetic and physical map to be bridged.

The isolation of the gene and studies of its spatial and temporal expression during development of the secondary palate should lead us to a greater understanding of mechanisms which may lead to the failure of closure of the neural crest. This mechanism can be compared with those which may cause sporadic cases in humans, or in animal models. There are also families known in which an X-linked form of spina bifida and anencephaly occur[17]; any homology in linkage between these families and the Icelandic family would be of interest.

Many disorders have both genetic and environmental elements. Polygenic and/or multifactorial disorders are complex to analyse, as has been elegantly shown for the role of LDL receptor defects in atherosclerosis[18]. Eventual cloning of the 'cleft palate' gene could be used as a starting point for the analysis of the genetic element of this developmental abnormality. For example, this gene may be a member of a multigene family[19] involved in the embryonic development of other tissues. Analysis of CP+A as a sex-linked single gene could provide a model for identifying other genes regulating processes in embryonic development whose expression is usually hidden in phenotypic, polygenic complexity.

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The molecular clock runs more slowly in man than in apes and monkeys

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The molecular clock hypothesis[1] postulates that the rate of molecular evolution is approximately constant over time. Although this hypothesis has been highly controversial in the past, it is now widely accepted[2–5]. The assumption of rate constancy has often been taken as a basis for reconstructing the phylogenetic relationships among organisms or genes and for dating evolutionary events[2–5]. Further, it has been taken as strong support for the neutral mutation hypothesis[5], which postulates that the majority of molecular changes in evolution are due to neutral or nearly neutral mutations[6]. For these reasons, the validity of the rate constancy assumption is a vital issue in molecular evolution. Recent studies[7–12] using DNA sequence data have raised serious doubts about the hypothesis. These studies provided support for the suggestion made from immunological distance and protein sequence data[13,14] that a rate slowdown has occurred in hominoid evolution, and showed, in agreement with DNA hybridization studies[15,16], that rates of nucleotide substitution are significantly higher in rodents than in man. Here, rates of nucleotide substitution in rodents are estimated to be 4–10 times higher than those in higher primates and 2–4 times higher than those in artiodactyls. Further, this study provides strong evidence for the hominoid slowdown hypothesis[13,14] and suggests a further rate-slowdown in hominoid evolution. Our results suggest that the variation in rate among mammals is primarily due to differences in generation time[8,16] rather than changes in DNA repair mechanisms[9]. We also propose a method for estimating the divergence times between species when the rate constancy assumption is violated.

Wu and Li[8] estimated that since the mammalian radiation the rate of synonymous substitution has been, on average, about two times higher in the rodent lineage than in the human and artiodactyl lineages. Because at the early stage of mammalian divergence the substitution rates in the three lineages should have been very similar, the rate differences noted by Wu and Li must have occurred in more recent times. To see if this is true we have compared the rates of synonymous substitution in primates, rodents and artiodactyls (Table 1). We used the method of Li *et al.*[17] to estimate the number of substitutions per synonymous site (K_S) and per nonsynonymous site (K_A) between two homologous genes. In this method, a nucleotide site is counted as one synonymous site if all possible changes at that site are synonymous, and $\frac{1}{3}$ or $\frac{2}{3}$ if one or two of the three possible changes are synonymous.

In the comparison between man and chimpanzee (Table 1), the synonymous rate varies considerably among the genes studied. This is probably largely due to chance effects, because the number of synonymous sites in each gene is small and the degree of sequence divergence is low. Further, the higher rates in the α1-, $^{G}\gamma$- and $^{A}\gamma$-globin genes might be partly due to gene conversions between the two α-globin genes[18,19] and between the two γ-globin genes[20,21]. Despite this possibility, the average rate for the five genes is only about 1.1×10^{-9} substitutions per synonymous site per year. In the comparison between man and the Old World (OW) monkeys, the synonymous rates are quite uniform over genes except for the insulin gene, and the average rate is about 2.3×10^{-9}. This is twice the average rate for the human–chimpanzee divergence, suggesting that OW monkeys have evolved two times faster than higher primates. The average rate (4×10^{-9}) between cow and goat seems to be substantially higher than that in higher primates, although the number of genes used is small. The rate estimated from the ruminant ε genes is even higher (4.8×10^{-9}), but these genes might have been involved in gene conversion events[22]. In the comparison between mouse and rat, except for the α-fetoprotein and albumin genes and some short sequences, the synonymous rates lie between 6×10^{-9} and 10×10^{-9}. It is seen that even the lower estimates of the synonymous rates in rodents are generally higher than the upper estimates in higher primates and monkeys. The intermediate estimate for the synonymous rate in rodents is 7 times higher than that in higher primates. This is similar to Britten's[9] estimate of a fivefold difference, derived mainly from DNA hybridization data. Considering the results in Table 1 and in Wu and Li[8] we suggest that the average synonymous rate in rodents is 4–10 times higher than that in higher primates, 3–6 times higher than that in monkeys, and 2–4 times higher than that in artiodactyls.

Table 1 Rates of synonymous substitution per site per year in primates, rodents and artiodactyls

Gene	No. of sites	Divergence (%)	Rate ($\times 10^{-9}$)
Man vs chimpanzee: divergence time used = 7 (5–10) Myr			
α1-Globin	108	2.8	2.0 (1.4–2.8)
α2-Globin	108	0.0	0.0 (0.0–0.0)
β-Globin	89	0.0	0.0 (0.0–0.0)
$^{G}\gamma$-Globin	101	3.0	2.1 (1.5–3.0)
$^{A}\gamma$-Globin	101	2.0	1.4 (1.0–2.0)
Total	507	1.6	1.1 (0.8–1.6)
Man vs OW monkeys: divergence time used = 25 (20–30) Myr			
α1-Antitrypsin	270	11.1	2.2 (1.9–2.8)
Erythopoietin	145	11.2	2.2 (1.9–2.8)
δ-Globin	104	10.4	2.0 (1.7–2.6)
Insulin	84	18.6	3.7 (3.1–4.7)
β-Globin (partial)	72	8.9	1.8 (1.5–2.2)
Metallothionein II	35	9.1	1.8 (1.5–2.3)
Total	710	11.6	2.3 (1.9–2.9)
Cow vs goat: divergence time used = 17 (12–25) Myr			
β-Globin	99	13.6	4.0 (2.7–5.7)
γ-Globin	105	13.5	4.0 (2.7–5.7)
Mouse vs rat: divergence time used = 15 (10–30) Myr			
Aldolase A	184	15.4	5.1 (2.6–7.7)
Creatine kinase M	251	17.2	5.7 (2.9–8.6)
Apoliproprotein E	201	17.4	5.8 (2.9–8.7)
THY-1	116	19.3	6.4 (3.2–9.6)
Metallothionein I	37	11.5	3.8 (1.9–5.7)
LDH-A	219	30.9	10.3 (5.2–15.5)
GPHA	58	30.8	10.3 (5.1–15.4)
ANF	107	20.4	6.8 (3.4–10.2)
Growth hormone	124	14.1	4.7 (2.4–7.1)
Prolactin	127	21.9	7.3 (3.7–11.0)
α-Actin	249	17.9	6.0 (3.0–8.9)
POMC	154	21.4	7.1 (3.6–10.7)
Thyrotropin, β	90	25.7	8.6 (4.3–12.8)
α-Fetoprotein	321	37.2	12.4 (6.3–18.9)
Albumin	274	36.7	12.2 (6.1–18.3)
Total	2,511	23.7	7.9 (4.0–11.9)

In the case of cow versus goat, the rate for β-globin is the average for the comparisons between cow β-globin gene and goat β^{A}- and β^{C}-globin genes. As divergence times are usually now well established, we have considered a range of estimates and an intermediate date for every pair of species compared. References: for the human–chimpanzee split[24,32]; the human/Old World monkey split[25,26]; the cow–goat split[29–31]; the mouse–rat split[4,38]. Data sources: α-globin[18,19]; β-globin[23,39]; erythropoeitin[40]; THY-1 (ref. 41); α-fetoprotein and albumin[33,42]; LDH-A (references in ref. 43); aldolase A[44,45]; creatine kinase M[46,47]; thyrotropinβ[23,48]. For other sequences, see ref. 23.

The results in Table 1 suggest a higher rate in artiodactyls than in primates. This is further supported by pseudogene data. For the α- and η-globin pseudogenes[11,23] (2,773 base pairs (bp)) the sequence divergence between human and chimpanzee is 1.7%, which corresponds to a rate of 1.2×10^{-9} if we assume

Table 2 Differences in the number of nucleotide substitutions per 100 sites between a primate (species 1) lineage and the human (species 2) lineage

Sequence	Sites* compared	NW monkeys		OW monkeys		Orangutan		Gorilla		Chimpanzee	
		K_{12}	$K_{13}-K_{23}$	K_{12}	$K_{13}-K_{23}$	K_{12}	$K_{13}-K_{23}$	K_{12}	$K_{13}-K_{23}$	K_{12}	$K_{13}-K_{23}$
η-Pseudogene	2,000	11.2	2.4† ± 1.1	7.3	2.1‡ ± 0.7	3.1	0.4 ± 0.4	1.4	0.5 ± 0.27	1.3	0.5 ± 0.27
Synonymous											
γ-Globin	100	13.6	−3.7 ± 6.8					0.0	0.0	2.5	1.8 ± 1.60
β-Globin	70			7.6	1.2 ± 6.0						
EPO	145			11.2	6.8 ± 5.9						
αl-AT	140			10.9	6.7 ± 8.9						
Insulin	84			18.6	−7.5 ± 6.9						
MT-II	35			9.1	3.6 ± 8.2						
α-Globin	105					6.9	3.2 ± 4.5			1.4	0.1 ± 2.96
Introns											
γ-Globin	900	11.6	3.1 ± 1.6					3.0	0.3 ± 0.66	2.7	0.0 ± 0.60
UT regions											
γ-Globin	255	15.3	3.9 ± 3.5					1.2	0.3 ± 0.75	0.8	0.0 ± 0.37
β-Globin	180			3.9	1.8 ± 1.5					0.0	0.0
α-Globin	140					4.3	1.1 ± 3.5			3.9	1.2 ± 3.41
mtDNA											
Fragment	895					18.0	0.9 ± 1.7	11.1	0.9 ± 1.31	9.4	1.4 ± 1.21
12S RNA	955							3.6	0.2 ± 0.80	3.8	0.4 ± 0.80
Total (nuclear)		11.6	2.6† ± 0.9	6.9	2.1‡ ± 0.6	3.2	0.4 ± 0.4	1.6	0.5† ± 0.24	1.3	0.3 ± 0.20
No. of sites		2,920		2,730		2,210		3,170		3,520	

The relative rate test[32] was used. In the case of pseudogenes and noncoding regions, the method of Jukes and Cantor[49] and Kimura and Ohta[50] was used to estimate the mean (K_{ij}) and variance (V_{ij}) of the number of substitutions per site between sequences i and j; $i, j = 1, 2$ or 3, where 3 refers to the reference species. Let $K_{03} = (K_{13} + K_{23} - K_{12})/2$, $P = 0.75[1 - \exp(K_{03}/0.75)]$ and $V_{03} = P(1-P)/[L(1-P/0.75)^2]$, where L is the number of sites compared. Then the variance of $K_{13} - K_{23}$ is given by $V_{13} + V_{23} - 2V_{03}$ (See ref. 8 for the derivation). In the case of synonymous substitutions, K_{ij} and V_{ij} were estimated by the method of Miyata *et al.*[51], and the variance of $K_{13} - K_{23}$ was computed according to the above procedure. In the case of New World (NW) monkeys versus human, the reference species was goat (η-pseudogene) or rabbit (γ-globin). In the case of Old World (OW) monkeys vs human, the reference species was owl monkey (η-pseudogene), cow and rabbit (β-globin), mouse (EPO and αl-AT), dog (insulin), or chinese hamster (MT-II). In the case of orangutan versus human, the reference species were owl monkey and rhesus monkey (η-pseudogene), rabbit (α-globin), or gibbon (mitochondrial (mt) DNA fragment). In the case of gorilla (or chimpanzee) versus human, the reference species were owl monkey, rhesus monkey and orangutan (η-pseudogene), spider monkey (γ-globin), orangutan (α-globin, mtDNA fragment and 12S RNA), or rhesus monkey (β-globin untranslated (UT) regions). When more than one species was used as a reference in a test, the smallest value for the variance of K_{13}-K_{23} was chosen; this still tends to overestimate the variance. 'Total' mean and variance were computed by using the reciprocal of the variance of K_{13}-K_{23} as weight. Data sources: γ-globin[7,10,20,21,52]; αl-antitrypsin (αl-AT)[23,53]; α-globin[23,54,55]; mtDNA fragment[56], and mt 12S RNA[57]. For the other sequences, see Table 1 and ref. 23.

* Approximate number of sites compared. † Significant at the 5% level. ‡ Significant at the 1% level.

Table 3 Mean (below diagonal) and standard error (above diagonal) of the number of nucleotide substitutions between species per 100 sites in the η-globin pseudogene

Species	Human	Chimpanzee	Gorilla	Orangutan	Rhesus	Owl monkey	Goat
Human	—	0.26	0.27	0.40	0.63	0.80	1.75
Chimpanzee	1.33	—	0.32	0.43	0.66	0.82	1.75
Gorilla	1.39	1.96	—	0.45	0.66	0.81	1.76
Orangutan	3.11	3.59	3.81	—	0.65	0.81	1.77
Rhesus	7.31	7.87	7.82	7.76	—	0.88	1.85
Owl monkey	11.16	11.64	11.58	11.46	13.26	—	1.83
Goat	35.11	35.11	35.59	35.68	38.20	37.52	—

Data from ref. 11 and references therein.

that human and chimpanzee diverged 7 Myr ago[24]. The divergence between the rhesus monkey and owl monkey η-globin pseudogenes[11] (1,967 bp) is 13.3%, corresponding to a rate of 1.7×10^{-9} if we assume 40 Myr for the divergence between the rhesus and owl monkeys[25-27]. For the comparisons between cow $\psi^3\beta$ and goat $\psi\beta^X$ and $\psi\beta^Z$ globin pseudogenes[23,28] (1,212 bp) the divergence is 9.1%. If we assume 17 Myr for the cow-goat split,[29-31], this corresponds to a rate of 2.7×10^{-9}, about 2.3 times higher than the rate in higher primates and 1.5 times higher than the rate in monkeys.

We now turn to the controversial issue of whether there has been a rate slowdown in hominoid evolution[4,13,14,24,32]. We use the relative rate test (see Table 2, footnotes). In Table 2, lineage 2 is always the human lineage whereas lineage 1 is either a monkey or an ape lineage. Therefore, a positive sign means that the human lineage has evolved more slowly and a negative sign means the opposite. It is striking that a negative sign appears in only two of the 30 comparisons (Table 2). Evidently, there is a general trend towards a slower rate in the human lineage. In the comparison between the New World (NW) monkey and the human lineages the difference in rate is significant (at the 5% level) in the case of the η-globin pseudogene and is close to significance in the case of γ-globin introns. The difference becomes highly significant when all sequences are considered together. The number of nucleotide substitutions per 100 sites is $7.1 = (11.6 + 2.6)/2$ in the NW monkey lineage and $4.5 = (11.6 - 2.6)/2$ in the human lineage. Thus, the rate is about 1.6 times faster in the former than in the latter lineage. In the comparison between the OW monkey and human lineages the difference in rate is highly significant even when only the η pseudogene is used. When all sequences are considered together, the rate is 1.9 (= 4.5/2.4) times faster in the OW monkey lineage than in the human lineage, similar to the factor of two derived above from synonymous substitutions (Table 1).

The rate in the human lineage seems also to be lower than those in the ape lineages (Table 2). In all the comparisons between an ape lineage and the human lineage, there is no case in which the human lineage has evolved faster. This is true for both nuclear and mitochondrial sequences. When all the nuclear sequences are considered together, the rates in the orangutan, gorilla and chimpanzee lineages are, respectively, 1.3, 1.9 and 1.6 times faster than the rate in the human lineage.

For η-globin, comparisons can also be made between apes and monkeys (Table 3). If we use owl monkey as a reference, it becomes clear that the rate is slower in the apes than in rhesus monkey because the distances from the former species to owl monkey are shorter than that from rhesus monkey to owl monkey. If goat is used as a reference, it also becomes clear that the rate is slower in the apes than in owl monkey. These results, together with those in Table 2, suggest that the substitution rate has become progressively slower in the human lineage since the separation of man from the OW monkeys.

As the rate constancy assumption is often violated, we need to consider the possibility of unequal rates among lineages when estimating the divergence time between species or genes. This can be done as follows, using the η-globin pseudogenes from primates as an example. Suppose that the branching order for the five primate species in Table 3 is as shown in Fig. 1, which is also the branching order inferred by either the maximum parsimony method[11] or Li's method[33]. The branch lengths are computed using a procedure that takes into account unequal substitution rates (Fig. 1 legend).

Suppose that we know the branching date for orangutan, say 12 Myr BP (ref. 34), and want to estimate the branching dates for gorilla and chimpanzee. The distances from node *b* to orangutan, gorilla and chimpanzee are 1.86, 1.95 and 1.91, respectively. Therefore, the rate constancy assumption holds approximately and the two branching dates are estimated to be $12\times 1.00/1.95=6.2$ Myr and $6.2\times 0.92/0.96=5.9$ Myr. Note that we avoid using the human lineage because the rate in that lineage has obviously been greatly reduced. As another example, suppose that we know the branching date for rhesus monkey, say 25 Myr (refs 25 and 26), and want to estimate the branchpoint for orangutan. Assuming that the substitution rate has been constant in the orangutan lineage since its separation from rhesus monkey, we estimate the orangutan branch point to be $25\times 1.86/(1.86+1.12)=15.6$ Myr, which is older than that suggested by Pilbeam[34]. The preceding assumption may not hold well but is obviously better than the assumption of equal rate in both the rhesus monkey and orangutan lineages because the length (4.78) for the former lineage is considerably longer than that (2.98) for the latter. Therefore, this approach can substantially reduce the effect of unequal rates among lineages. Using this approach and assuming that owl monkey (the NW monkeys) branched off 35-45 Myr ago[25-27], we estimate that the rhesus monkey (the OW monkeys) branchpoint is 24.0-30.9 Myr ago, the orangutan branchpoint is 15.0-19.3 Myr ago, the gorilla branchpoint is 7.7-9.9 Myr ago and the chimpanzee branchpoint is 7.4-9.5 Myr ago. These estimates are close to the dates proposed by Andrews[35] and Pilbeam[25].

Instead of differences in generation time or, more precisely, in the number of germline DNA replications per year[8,16], changes in DNA repair mechanisms[9] have recently been proposed as the primary cause of rate differences among mammals. We do not exclude the possibility that the DNA repair system is more effective in man than in rodents, but we do not find it necessary to invoke such changes to explain the large differences in substitution rate among mammals. The generation time in rodents (mouse and rat) is about 100 times shorter than that in man, so the number of germline DNA replications per year in rodents could be 10 times higher than that in man[36]. Such a large difference in the number of germline DNA replications per year would be sufficient to explain the approximately sevenfold difference in synonymous substitution rate between rodents and humans, on the assumption that the mutation rate per DNA replication is constant. Rhesus monkeys reproduce as early as age 3, whereas humans do not reproduce until age 12 or older. Again the difference in DNA replication number is potentially sufficient to account for the approximately twofold difference in rate of silent substitution between the rhesus monkey and human lineages. The generation time in artiodactyls is much closer to that in primates than to that in rodents, which is consistent with our observations on relative substitution rates.

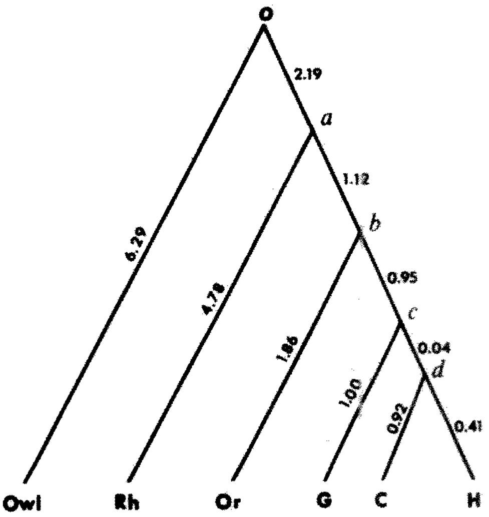

Fig. 1 A phylogenetic tree for the primates. The branching sequences were inferred by applying Li's method[33] to the distances between primate species in Table 3; the tree is also the maximum parsimony tree[11]. The number on each branch is the number of nucleotide substitutions per 100 sites. Using goat as a reference, we find that the distance from node *o* to rhesus monkey is $38.20-37.52=0.68$ longer than that from node *o* to owl monkey (Table 3). Because the distance between owl monkey and rhesus monkey is 13.26, the distance from node *o* to owl monkey is $(13.26-0.68)/2=6.29$ (Fig. 1) and that from node *o* to rhesus monkey is 6.97. Similarly, using owl monkey as a reference, we find that the distances from node *a* to rhesus monkey and orangutan are 4.78 and 2.98, respectively. Therefore, the distance from node *o* to node *a* is $6.97-4.78=1.12$. The distance from node *b* to orangutan was obtained using owl monkey and rhesus monkey as references and the other branch lengths were obtained using owl monkey, rhesus monkey and orangutan as references. Owl, Rh, Or, G, C and H denote owl monkey, rhesus monkey, orangutan, gorilla, chimpanzee and human, respectively.

The rate constancy assumption was an important argument in the formulation of the neutral mutation hypothesis[37] and has always been taken as strong support for it[5]. Violation of this assumption, however, should not be taken as evidence against the hypothesis, for a constant substitution rate would be predicted only if the mutation rate remains constant. Indeed, a serious criticism of the rate-constancy argument has been that the approximate constancy seen in protein sequence data is in terms of chronological time rather than generation number, but mutation rates in different organisms are more nearly comparable when measured in generations than in absolute time units. Therefore, the finding that the rate of nucleotide substitution is higher in short-living organisms, such as rodents, than in long-living organisms, such as humans, is actually more in line with the neutral mutation hypothesis than if the rates are equal for all organisms.

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Accelerated evolution in the reactive centre regions of serine protease inhibitors

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The serine protease inhibitors (serpins) are a family of proteins that function to control the action of serine proteases in many diverse physiological processes. The functional region or reactive centre of these inhibitors is near the C-terminal end and is an exposed site that acts as a bait for the appropriate serine protease to recognize and covalently bind. The specificity of the inhibitor is determined, at least in part, by a single amino acid that resides in this region at the P_1 position[1]. We show here that following a gene duplication event the reactive centres of three related rodent protease inhibitors have diverged from each other at unprecedented rates. This has resulted in proteins with different predicted specificities and we postulate that these changes were fixed by positive darwinian selection and that the most likely selective forces are extrinsic proteases, namely those used by parasites to facilitate their spread throughout the host.

We have isolated complementary DNA clones for several of the major rodent protease inhibitors that are synthesized in the liver and subsequently secreted into the plasma. One of these has been identified as the cDNA encoding contrapsin[2] which is most closely related to human α_1-antichymotrypsin (α_1-achy). Despite having a high degree of similarity, these two inhibitors have different protease specificities: contrapsin is an inhibitor of trypsin-like proteases[3,4], whereas α_1-achy is an inhibitor of chymotrypsin-like proteases[5-7]. The difference in specificity between these two proteins has been attributed to the dramatic degree of non-homology within the short stretch of amino acids encompassing the reactive centre region and particularly to the resulting change in the P_1 amino acid[2]. To explain this fundamental difference in protease specificity we have investigated these genes in rodents further. Although humans have only one α_1-achy gene (unpublished data) we have shown that mouse and rat have multiple contrapsin-related genes[8]. In the mouse they are arranged in a tightly clustered array on chromosome 12 designated the *Spi-2* locus. The possibility therefore arose that these related genes have evolved to acquire different functions, one of them equivalent to human α_1-achy.

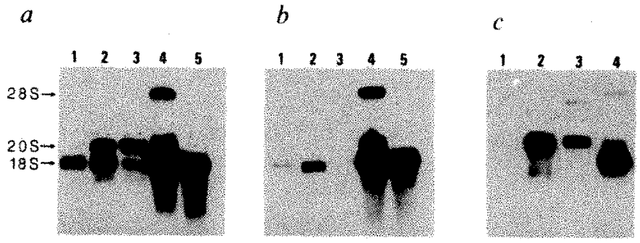

Fig. 1 Northern blot analysis of mouse and rat liver poly(A)$^+$RNA hybridized with different contrapsin DNA probes. The probes used were *a*, the full length contrapsin cDNA; *b*, the 3′ end of the contrapsin cDNA; *c*, a 16-nucleotide oligonucleotide. Hybridization conditions in *c* were 5 × SSC at 37 °C overnight and washed in 5 × SSC at 42 °C. Lanes 1-3, rat RNAs; lane 4 BALB/c mouse RNA and lane 5, C57BL/6 mouse RNA. RNA was isolated from rats which were (lane 1) normal, (lane 2) treated with bacterial lipopolysaccharides to induce an inflammatory reaction or (lane 3) treated with dexamethasone. RNA extractions[24] and Northern blot analysis[25] were performed as described.

The messenger RNA isolated from mouse and rat liver contains at least two different contrapsin-related mRNA transcripts of ~1.8 kilobases (kb) (18S) and 2.2 kb (20S)[8,9]. (A third mRNA of 5 kb has not been well characterized.) The 2.2 kb mRNA is induced in the rat and in some mouse strains during an inflammatory response and also upon treatment with dexamethasone (Fig. 1*a* and ref. 9). We shall refer to these as '*spi-2*' mRNAs after the name of the genetic locus in the mouse[8]. The 1.8 kb mRNA species will be referred to as the *spi-2.1* mRNA in the rat, but as contrapsin mRNA in the mouse, where an activity has been established for the gene product[2,3]. The 2.2-kb mRNA will be referred to as the *spi-2.2* mRNA in both species.

In the rat, the *spi-2* RNAs are likely to be encoded by different genes (Fig. 1). Two DNA probes representing different regions of the contrapsin mRNA differentially hybridize to the rat *spi-2* mRNA species. A probe containing the 3′ portion of the mouse contrapsin cDNA hybridizes specifically to the rat *spi-2.1* mRNA (Fig. 1*b*), whereas a synthetic oligonucleotide corresponding to a region in the middle of the mRNA hybridizes specifically to the rat *spi-2.2* mRNA (Fig. 1*c*).

```
Region 1
       1
  con GTG GTG CTG GTG AAT TAC ATC TAC TTT AAA GGC AAA TGG AAG ATA TCC TTT
  2.1 C-- --- --- --- --C --T CT- CTA --- --- --- --- --- --- G-C C-- ---
  2.2 A-- --A --- --- --- --- --- --- --- --- --- --- --- --- G-G C-T ---
α-ac  A-- --C --- --- --- --- --- -T- --- --- -C- --- --- G-- --G C-- ---
     52
  con GAC CCC CAG GAC ACA TTT GAG TCT GAG TTC TAC TTG GAT GAG AAG AGA TCT
  2.1 A-- --T A-T --- --- --- --- --- --- --- --- --- --- --- --- --G ---
  2.2 G-- --T -G- --- --- --C C-- --- --- --- --- -CT -GC A-A -G- --G C--
α-ac  --- --- --A --T --T CA- C-- --A AG- --- --- --- AGC A-- --A -AG -GG
    103
  con GTG AAG GTT CCC ATG ATG AAA ATG AAG TTA CTG ACC ACA CGC CAC TTC CGT
  2.1 --- --- --G --- --- --- --- --T --- GA- G-A --- --- -C- T-T G-- --G
  2.2 --- --A --G --- --- --- --G C-T G-- GAC --- --- --- -C- T-- G-- --G
α-ac  --A -T- --G --- --- --- -GT T-- C-T CAC --- --T -T- -GT T-- --- --G
    154
  con GAT GAG GAG CTA TCG TGC TCT GTG TTG GAG CTG AAG TAC ACA GGA AAT GCC
  2.1 --- --- --A --C --C --- --- --- --- --- --- --- --- --- --- --- ---
  2.2 --- --- --- --G AAC --- A-- --T G-- --- --- --- --- --- --- --- ---
α-ac  --C --- --- --G --C --- A-C --- G-- --- --- --- --- --- --C --- ---
    205
  con AGC GCC CTG CTC ATC CTC CCT GAC CAG GGC AGG ATG CAG CAG GTG GAA GCC
  2.1 --- --- --- T-T --- --- --- --- --A --- -A- --- --- --- --- --- T--
  2.2 --- --- --- T-T --- --- --- --- --- --- -A- --- --- --- --- --- ---
α-ac  --- --A --C T-- --- --- --- --T --A -A- -A- --- G-- G-A --- --- ---
    256
  con AGC TTA CAA CCA GAG ACC CTG AGG AAA TGG AGG AAA ACT TTG TTT CCC AGC
  2.1 --- --G --- --- --- --- --- -A- --G --- -A- G-C T-- --- A-C --T ---
  2.2 --- --G --- --- --- --- --- --- -G- --- -A- G-C T-- C-C AGG --- ---
α-ac  -TG C-G -TC --- --- --- --- -A- CGG --- --A G-C T-- C-- GAG TT- --A
    307
  con CAA ATA GAG GAG CTA AAC CTG CCC AAG TTC TCC ATC GCT AGT AAC TAC AGG
  2.1 ATT --- A-T --T --C CG- A-- --- --- --- --- --- T-C -CA G-- --- --T
  2.2 ATG --- --T --- --C T-- --- --- --- --- --- --- T-- GC- G-- --- -AC
α-ac  G-G --- -GT --- --C T-- --- --A --- --T --- --- T-G --G G-- --T -AC
    358
  con CTG GAG GAG GAT GTC CTT CCA GAA ATG GGG ATT AAG GAA GTC TTC ACC GAA
  2.1 --A     A-A --G --- --- --- --G C-- --- --- --- A-- --- --- T-- C-G
  2.2 ---     --- --C --- --- --- --G C-- --C --C --A --- -CT --- T-- AC-
α-ac  ---     A-C --C A-A --- -TC C-G -TG --C --- G-- --- -C- --- --- AGC
    409
  con CAA GCT GAC CTA TCT GGG ATC ATA GAA ACC AAG AAA CTG AGT GTG TCT CAG
  2.1 --- --- --T --G AG- A-- --- -C- -GG --- --- G-T --- TA- --- --- --A
  2.2 --G --- --- --G --- --- --- -C- -GG GAT --- G-C --- -TG --- --- G--
α-ac  A-G --- --- --G --A --- --- -C- -GG G-- -G- --C --A GCA --C --C ---
    460
  con GTG GTC CAC AAG GCT GTG CTG GAT GTG GCT GAG ACA GGC ACA GAA GCA GCT
  2.1 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- A--
  2.2 --- --- --- --- --- --T --- --- --- --- --- --- --- --- --- --- --C
α-ac  --- --- --T --- --- --- --T --- --A TT- --- GAG --- --- --- --- T--
    511              ———————— Region 2 ————————————————
  con GCT GCC ACA GGG GTT ATT GGT GGC ATT CGT AAG GCC ATA TTA CCA ••• •••
  2.1 --- --T --- --- --C GC- ACA -T- --A --- -GA CAA CCT CGA ACT ••• •••
  2.2 --- --- --- --- --C -AA TT- -TT CCA GTG TCT --A -A- C-G GAC CCT CTG
α-ac  --- --- --- --A --C -AA ATC AC- C-C -T- TCT --A T-- GTG GAG ACA AGG
    562 ——————+——————————————— Region 3 ———————————————
  con ••• GCT GTG CAT TTC AAC AGG CCA TTC CTG TTT GTT ATC TAT CAC ACA AGT
  2.1 ••• ••• C-- A-- --- --- CA- --- --- A-- G-G T-- --- AC- G-- -TG GA-
  2.2 ••• AT- ATA GC- --- G-- C-- --T --- --- A-G A-- --- -C- G-- --- GAA
α-ac  ACC AT- --- -GT --- --- --- --C --- --- A-G A-C --T GTC -CT --- GAC
    613 ——————————————————————————————————————————
  con GCT CAG AGT ATC CTC TTT ATG GCC AAA GTC AAT AAC CCC AAG TAG ACC TAA
  2.1 AG- --- TC- --- --- --- G-T --- --- A-- -C- --- --- -GT --A G-- C--
  2.2 A-- GCA -TA GCT -C- --- T-- --- --G A-A TT- --- --- --A -GA GAT -CG
α-ac  A-C --- -AC --- T-- --C --- AG- --- --- -CC --T --- --- C-A G-- --G
    664
  con •GCTCACCAAGTTCTCATGGTTCTGTCCAGGGTTCTGGGACAAAGTCTGCATG•TGGATCTCTATAT
  2.1 A----C-----A---G-CA------C-----ACC----A•-TG--C-------•--------G---
  2.2 -A--TC-----AGT-G---C----CCT•••••GAG-CA•TTT--C---TC--•---G-----G-G-
α-ac  A---TG---TCA-GCAG---GG--C--AGT••AA--AACTTG--AA----A-C-----GC--GGG-
    731
  con ACATCCTGTCATTCAAGCTGTGATTGGCT••GTTGCAAAGTTGGCTTAGAC•AGCTACATTAACTA
  2.1 -TG-----A------T-T
  2.2 G---TT--G-T-CT-T---C--------C••A-G--•GGCA--C--••-GA•T-AG---G------
α-ac  CTC-G••-G--C••---- C-••••---CCCT--•----CGAG----CAT-G-AT-TG••TGGGG--G
    798
  con TCTCTGTGGTCAGT
  2.2 A--G---AA••----CCTCATGT•ACAGACGCCTGTGCAGAGTCGCTGCCATGCTCCCAAACTTCTTGG
α-ac  ---GCT-AT•-CT-GGAAGGTGACAGCGATT
    865
  2.2 TACCACTAGCTCATATTTCTGAGCCTAAAATTTGCCTTTCCCCTGCCCTTGCTCTCTCTCCCCCGTA
    932
  2.2 TCTGCCTCAACCCAGAAGCCAGGGCCCCATCATAGGTTGTCTCAGTCCCAACTTAGGTCTAGTTATA
    999
  2.2 TCTTCCCTTCAGCGTTGCTGTCTTGATGGGGCTGTGCGCG
```

Fig. 2 Nucleotide sequence comparisons of rodent and human *spi-2* cDNAs. The sequences represent the 3′ half of the mRNA. The contrapsin and human α-achy sequences were previously reported[5]. All sequences are compared to mouse contrapsin cDNA, with only nucleotide changes shown in the other three sequences. The coding region sequences are divided into three regions by the degree of homology of the four sequences. Stop codons are boxed, and gaps are designated by dots. Sequences were determined by the chain termination method of Sanger[26]. Mouse contrapsin is designated here as con, the two rat *spi-2* genes are 2.1 (18S mRNA) and 2.2 (20S mRNA) and human α_1-achy is α-ac. The contrapsin 3′ end probe (Fig. 1*b*) starts at nucleotide 510 and continues to the end; the 16-nucleotide oligomer (Fig. 1*c*) starts at nucleotide 7.

To isolate cDNAs corresponding to the rat *spi-2* mRNAs, we screened liver cDNA libraries using these specific probes, and sequenced a cDNA from each of the two classes. In Fig. 2, these data are compared to the contrapsin cDNA and the human α_1-achy cDNA sequence data previously reported[2]. Comparison of these four sequences reveals a striking trend in the degree of homology such that the predicted coding regions of the cDNAs can be subdivided into three regions (Fig. 2). Region 1 includes the 5′ 516-bp portion of the cDNAs representing the middle of the mRNA (Fig. 2) and is the most-conserved region. Region 2 is a shorter stretch of DNA encompassing the reactive-centre region. In contrast to region 1, there is complete breakdown in sequence homology and in some cases (*spi-2.1* versus *spi-2.2* and contrapsin versus *spi-2.2)* no more similarity is observed than would be expected (~30%) between two unrelated cDNAs (Table 1). Region 3 encodes the C-terminal portion of the proteins and shows an intermediate level of nucleotide sequence similarity.

In Fig. 3, the predicted amino-acid sequences of the four inhibitors are compared and amino acids that are conserved in three of the four proteins are boxed. Again, in region 2, the homology is reduced quite dramatically and breaks down abruptly at the boundaries. Nowhere else in the molecule can a stretch of comparable length approaching this degree of divergence be found.

Simple calculations show that region 2 of the rodent protease inhibitors has evolved rapidly at both synonymous and nonsynonymous positions of the codon. To evaluate the true significance of the data, however, we have used the method of Li *et al.*[10] to calculate the number of substitutions at both types of site in these three protein regions. This method gives a K_A (number of substitutions per nonsynonymous site) and K_S (number of substitutions per synonymous site) value for the genes compared. The K_A values for all functional genes analysed are lower than K_S values, but show a range of values depending on the degree of functional constraints placed on the protein. Because synonymous positions are subject to far fewer functional constraints, if any, the values for K_S are more constant.

Table 1 Nucleotide sequence similarity and numbers of substitutions between *Spi-2*-like genes

Nucleotide sequence homology (%)

Region	con vs *spi-2.1*	con vs *spi-2.2*	*spi-2.1* vs *spi-2.2*	Con vs α_1-achy	*spi-2.1* vs α_1-achy	*spi-2.2* vs α_1-achy
1	84	83	84	74	75	77
2	33	28	24	41	31	53
3	75	64	65	69	60	57

No. of substitutions per synonymous (K_S) and non-synonymous (K_A) site between genes

	con vs *spi-2.1*		con vs *spi-2.2*		*spi-2.1* vs *spi-2.2*		con vs α_1-achy		*spi-2.1* vs α_1-achy		*spi-2.2* vs α_1-achy	
Region	K_S	K_A	K_S	K_A	K_S	K_A	K_S	K_A	K_S	K_A	K_S	K_A
1	0.35	0.14	0.38	0.16	0.42	0.13	0.76	0.24	0.69	0.25	0.70	0.18
	(0.07)	(0.02)	(0.07)	(0.02)	(0.08)	(0.02)	(0.12)	(0.03)	(0.12)	(0.03)	(0.11)	(0.02)
2	NA	1.38	NA	1.42	0.88	NA	2.24	0.99	1.00	NA	0.79	0.62
		(0.56)		(0.59)	(0.86)		(2.90)	(0.34)	(0.59)		(0.52)	(0.23)
3	0.26	0.44	1.33	0.39	0.95	0.50	1.63	0.27	NA	0.45	NA	0.40
	(0.15)	(0.11)	(0.99)	(0.10)	(0.51)	(0.12)	(0.99)	(0.08)		(0.11)		(0.10)

Values in parenthesis are standard errors; NA, not applicable (no meaningful estimate can be obtained). Abbreviations for cDNAs are as follows: con, mouse contrapsin gene; α_1-achy, human α_1-anti-chymotrypsin gene; *spi-2.1* and *spi*-2.2, rat *spi-2* mRNAs (see text).

The K_S values should more closely reflect the rate of divergence of two genes.

Table 1 also shows K_S and K_A values for each of the six possible comparisons for the *spi-2* like genes subdivided into the three regions (W.-H. Li kindly carried out this analysis). From these values we draw the following conclusions. First, in region 1 as in all protein-coding regions analysed so far, the K_S value is several times greater than the K_A value. The average ratio of K_S/K_A for all possible comparisons of region 1 in the rodent protease inhibitor genes is 2.7. The average ratio of K_S/K_A for 37 mammalian genes analysed by this method is 5.3. However, in the more rapidly evolving proteins such as interferon-α_2, β-globin and relaxin this ratio is between 2 and 3. Therefore serine protease inhibitors in region 1 compare with other known proteins; albeit the proteins that evolve most rapidly. Secondly, K_A for region 2 equals or exceeds the K_S value of region 1. In some comparisons (mouse contrapsin gene versus either rodent gene), the K_A values for region 2 are four times greater than the K_S values for region 1. This high degree of substitution in region 2 indicates that the part of the gene containing the reactive centre has undergone accelerated evolution; a process postulated to occur in genes following a duplication event[11-13]. Note that the values for region 2, because of the high degree of divergence, are in some cases not calculable. Where they are calculable, conservative estimates are stated: the actual values could be much greater. Finally, the K_A values in region 3, although lower than in region 2, reveal that this segment of the genes is also evolving at an accelerated rate, because the numbers of substitutions in all but one comparison (contrapsin versus α_1-achy) are appreciably higher than the K_A values for region 1 and, in the rodent comparisons at least, as high as the K_S values of region 1.

To determine the rate at which these genes have evolved, and to allow comparisons with rates for other genes studied, the evolutionary history of this gene family must be established. We suggest, that the genes that code for the *spi-2* mRNAs are a result of a duplication event that occurred before mouse and rat divergence and also that the mouse contrapsin and rat *spi-2.1* genes are orthologous as are the mouse and rat *spi-2.2* genes. These conclusions are drawn for the following reasons. (1) Messenger RNAs of 18S (*spi-2.1*) and 20S (*spi-2.2*) are found in both species (Fig. 1); (2) there is a high constitutive level of the *spi-2.1* mRNA in both species; (3) the *spi-2.2* mRNA is induced by inflammation and dexamethasone in at least some strains of mice[8] and in rats (Fig. 1); (4) a 3'-end DNA probe specific for the rat *spi-2.2* mRNA hybridizes only to the mouse *spi-2.2* mRNA (data not shown); a similar probe specific for mouse contrapsin hybridizes only to rat *spi-2.1* (Fig. 1*b*). This is supported by the degree of nucleotide similarity for the *spi-2.1* genes in region 3 (Table 1, top); (5) attempts to detect other *spi-2*-like mRNA species in mouse or rat and to isolate another class of related cDNA clones from rat-liver libraries have failed. By definition, the orthologous genes have been separate only since mouse and rat divergence. The date of this divergence is controversial and estimates range from 10 to 30 Myr ago, although 15 Myr is considered a reasonable estimate (W.-H. Li, personal communication). To determine when the gene duplication event occurred, we have taken region 1 as representing that part of the gene evolving comparably to other rodent genes analysed. Comparing the K_S value for region 1 of the rat *spi-2.1* and *spi-2.2* genes to the K_S value of the putative orthologous genes, mouse contrapsin and rat *spi-2.1*, we predict that the duplication occurred some 3 Myr before mouse-rat divergence. The rate of divergence of two orthologous genes is defined as the number of substitutions occurring per site per year; a value of 46×10^{-9} is obtained for the nonsynonymous sites in region 2 of the mouse contrapsin and rat *spi-2.1* comparison ($23-69 \times 10^{-9}$ taking the range of dates of 10-30 Myr). Analysis of many genes has shown that the most rapidly evolving bases are those in synonymous positions of codons, 3' untranslated regions, 3' flanking regions of genes and pseudogenes[14]. Pseudogenes evolve most rapidly, although the differences in rates amongst the above categories is on average <30%. Pseudogenes are believed to be subject to no functional constraints and should accumulate substitutions at a rate that reflects the mutation rate. When 14 different orthologous pairs of genes were compared between mouse and rat the average rate of substitution at synonymous sites was found to be 8×10^{-9} per base per year (W.-H. Li, personal communication) For two mouse pseudogenes ($\psi\alpha 3$ globin and immunoglobin VH108B) the rate of divergence was 6×10^{-9} per base per year[15]. Thus in region 2 the rate of substitution at nonsynonymous positions is 6-8 times greater than that for the most rapidly evolving stretches of DNA.

Considering the unlikely event that rat *spi-2.2* and contrapsin are orthologous or indeed none of the pairs of genes we have compared is orthologous, the conclusion of an accelerated rate of divergence in the reactive centre region is still inescapable. If we further postulate that region 2 is actually a non-functional region of the protein and therefore is accumulating substitutions at an unconstrained rate (at the rate of pseudogenes) then these genes would have become separated some 100-150 Myr ago: unlikely because regions 1 of the human and rodent inhibitor genes are diverging at close to the rate commonly found for a number of genes.

The substitutions in the reactive-centre regions of these proteins have resulted in inhibitors with varying specificities. Aligning the reactive centres of the two rat genes, *spi-2.1* and *spi-2.2*, to the human sequence in which the P_1 position is known[16]

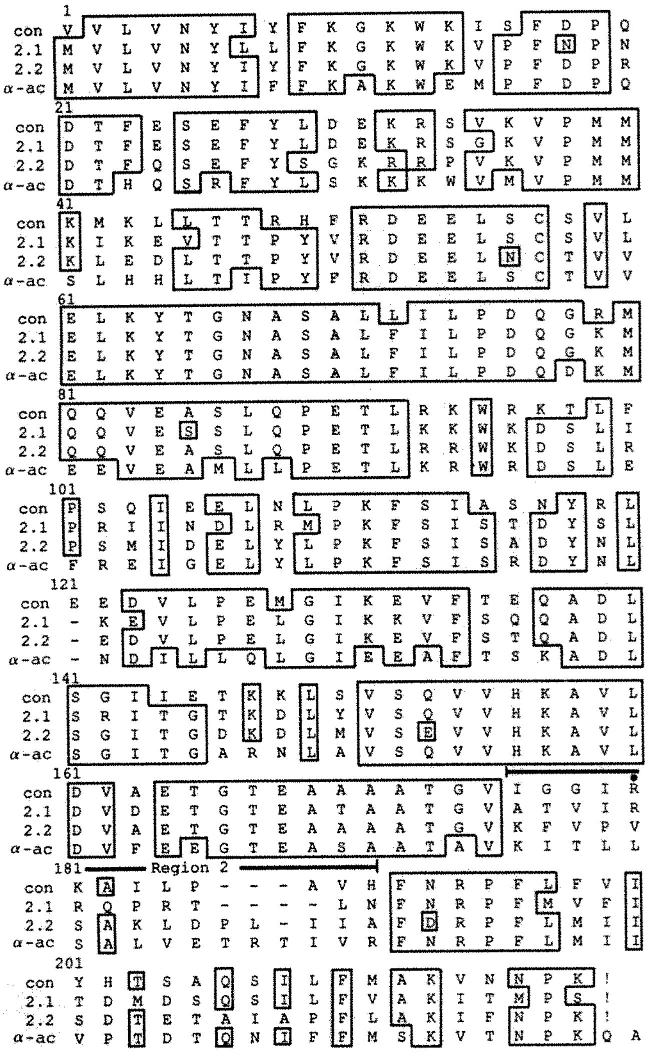
1
con V V L V N Y I Y F K G K W K I S F D P Q
2.1 M V L V N Y L L F K G K W K V P F N P N
2.2 M V L V N Y I Y F K G K W K V P F D P R
α-ac M V L V N Y I F F K A K W E M P F D P Q
21
con D T F E S E F Y L D E K R S V K V P M M
2.1 D T F E S E F Y L D E K R S G K V P M M
2.2 D T F Q S E F Y S G K R R P V K V P M M
α-ac D T H Q S R F Y L S K K K W V M V P M M
41
con K M K L L T T R H F R D E E L S C S V L
2.1 K I K E V T T P Y V R D E E L S C S V L
2.2 K L E D L T T P Y V R D E E L N C T V V
α-ac S L H H L T I P Y F R D E E L S C T V V
61
con E L K Y T G N A S A L L I L P D Q G R M
2.1 E L K Y T G N A S A L F I L P D Q G K M
2.2 E L K Y T G N A S A L F I L P D Q G K M
α-ac E L K Y T G N A S A L F I L P D Q D K M
81
con Q Q V E A S L Q P E T L R K W R K T L F
2.1 Q Q V E S S L Q P E T L K K W K D S L I
2.2 Q Q V E A S L Q P E T L R R W K D S L R
α-ac E E V E A M L L P E T L K R W R D S L E
101
con P S Q I E E L N L P K F S I A S N Y R L
2.1 P R I I N D L R M P K F S I S T D Y S L
2.2 P S M I D E L Y L P K F S I S A D Y N L
α-ac F R E I G E L Y L P K F S I S R D Y N L
121
con E E D V L P E M G I K E V F T E Q A D L
2.1 - K E V L P E L G I K K V F S Q Q A D L
2.2 - E D V L P E L G I K E V F S T Q A D L
α-ac - N D I L L Q L G I E E A F T S K A D L
141
con S G I I E T K K L S V S Q V V H K A V L
2.1 S R I T G T K D L Y V S Q V V H K A V L
2.2 S G I T G D K D L M V S E V V H K A V L
α-ac S G I T G A R N L A V S Q V V H K A V L
161
con D V A E T G T E A A A A T G V I G G I R
2.1 D V D E T G T E A T A A T G V A T V I R
2.2 D V A E T G T E A A A A T G V K F V P V
α-ac D V F E E G T E A S A A T A V K I T L L
181 Region 2
con K A I L P - - - A V H F N R P F L F V I
2.1 R Q P R T - - - - L N F N R P F M V F I
2.2 S A K L D P L - I I A F D R P F L M I I
α-ac S A L V E T R T I V R F N R P F L M I I
201
con Y H T S A Q S I L F M A K V N N P K !
2.1 T D M D S Q S I L F V A K I T M P S !
2.2 S D T E T A I A P F L A K I F N P K !
α-ac V P T D T Q N I F F M S K V T N P K Q A

Fig. 3 Comparison of the predicted protein sequences for all four *spi-2* genes. Amino acids the same for three of the four proteins are boxed. Only region 2 is designated. Amino acids are shown in single-letter code. The P_1 amino-acid position is indicated by a dot.

allows predictions of the inhibitory activities. The P_1 amino acid predicted in *spi-2.1* is an arginine whereas in *spi-2.2* a valine lies at this position. A positively charged amino acid at this position usually results in an inhibitor with anti-tryptic activity[17], whereas a valine results in anti-elastinolytic activity.[18,19]. The mouse contrapsin, like the rat *spi-2.1* protein, contains a positively charged amino acid at the P_1 position. It remains to be determined how the amino acids in the surrounding area affect the physiological function of these inhibitors. However, it has been shown by X-ray crystallography of a model system[20] that the four amino acids N-terminal to the P_1 site are in direct contact with the complexed protease.

How can we account for the high rate of substitution in the reactive centre region of these proteins? We postulate that in region 2 of the protein, nucleotide substitutions are being rapidly fixed by positive darwinian selection. Changes that advantageously affect the activity of the protein would be fixed more rapidly in a population than would neutral mutations. It is unlikely that these inhibitors are acting exclusively, or at all, upon indigenous proteases as this would require rapid co-evolution of the target protease. We feel it is more likely that these inhibitors are acting upon exogenous proteases brought in by infectious agents upon wounding or parasitic infection. It is known that parasites such as *Schistosoma mansoni*[21], and bacteria such as *Pseudomonas*[22] synthesize serine proteases that act as virulence factors to increase the efficiency of infection. Therefore, inhibition of these types of proteases would provide an appreciable selective advantage to the host. However, it is unlikely that all of the substitutions that have arisen in the *spi-2* genes have been selected for. We suggest that many of the mutations have been carried along with the advantageous substitutions by the process called 'hitchhiking'[23]. This may explain the overall rapid divergence (low K_S/K_A ratio) of these proteins. The molecular mechanism by which the rapid rate of divergence in region 2 has occurred may be elucidated by the study of more closely related rodent species.

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Developmentally controlled expression of a gene associated with self-incompatibility in *Nicotiana alata*

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In many flowering plant species, a system of self-incompatibility, typically controlled by a single gene with multiple alleles (S-gene)[1], enables individual plants to recognize and reject their own pollen. In gametophytically determined systems, the growth of self-pollen tubes is arrested in the style or occasionally the ovary. The expression of this self-incompatibility is developmentally regulated, being strongest in mature flowers and weak in immature styles[1,2]. We have used a complementary DNA clone, believed to encode the S_2-allele of *Nicotiana alata*[2,3], to detect S_2 messenger RNA by *in situ* hybridization to sections. We report here that expression of the gene occurs in the stigma and throughout the secretory tissue, but not in other parts, of mature pistils. In

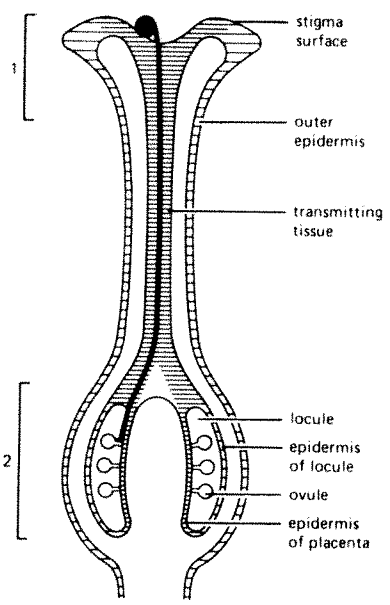

Fig. 1 Diagram of compatible pollination of a mature pistil of *Nicotiana alata*. The secretory epidermis of the stigma, transmitting tract of the style and the epidermis of the placenta are indicated by close cross-hatching; these are the tissues which hybridize with S_2 cDNA and correspond to the path taken by the pollen tube before entering the ovule. The outer epidermis of the pistil and the epidermis of the locule originate from the same cell line as the transmitting tissues and are indicated by wide cross-hatching. Area 1, sections through the stigma and style as in Fig. 3. Area 2, sections through this ovary region as in Figs 2 and 4.

immature flowers, S_2 mRNA is confined to the proliferated epidermis of the stigma. We conclude that S_2-gene expression correlates well with the expression of incompatibility.

During maturation of pistils of the Solanceae, the epidermal layer (layer LI, ref. 4) initially divides to form the epidermis of the placenta and the ovary locule as well as the initials of the stigma surface secretory tissue and the transmitting tissue. This is followed by cell division to form the receptive stigma, and cell division and elongation to form the transmitting tissue of the style. Development of this tissue proceeds from the top to the base of the style[4]. Thus, there is a continuous secretory tissue connecting the stigma surface, the style transmitting tract and the inner epidermis of the ovary (which includes both the epidermis of the placenta and the outer epidermis in each locule) (Fig. 1). During a compatible pollination, pollen received on the stigma surface germinates to produce pollen tubes which grow extracellularly through the transmitting tissue of the style, then over the surface of the placental epidermis (Fig. 2) into the micropyles of the ovules. The sperm cells are then released from the tube tip to effect the double fertilization characteristic of flowering plants. In this paper, we report the use of hybridization histochemistry to show the development of tissue-specific expression of the gene corresponding to S_2 cDNA (designated the S_2-gene) of *N. alata*.

We reported previously that we were unable to detect hybridization of the S_2 cDNA to mRNA isolated from immature styles using Northern analysis[2]. More recently, we have shown by primer extension experiments, that the immature pistil has very low levels of S_2 mRNA (M. A. Anderson, unpublished data). Hybridization of the S_2 cDNA to longitudinal sections of immature pistils (Fig. 3) showed that hybridization was restricted to two well-defined sites in the stigma which correspond to the proliferated stigma epidermis. There was no detectable hybridization to the transmitting tract or ovary of immature pistils. In *N. alata* (S_2S_3), self pollen tubes are inhibited after growing through the upper third of the style. Thus, pollen-tube arrest does not occur immediately after initial contact with the female

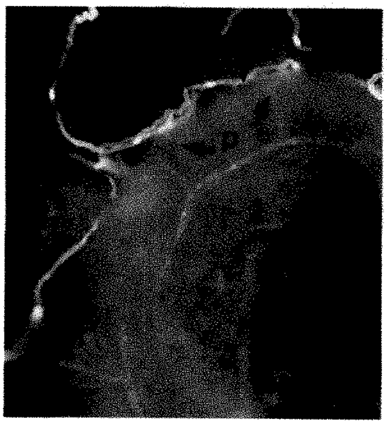

Fig. 2 Fluorescence micrograph of a hand section through the ovary stained with aniline blue fluorochrome (Biosupplies Australia) showing pollen tubes (pt) growing over the surface of the placenta (p) toward the ovules (o). Bar, 50 μm.

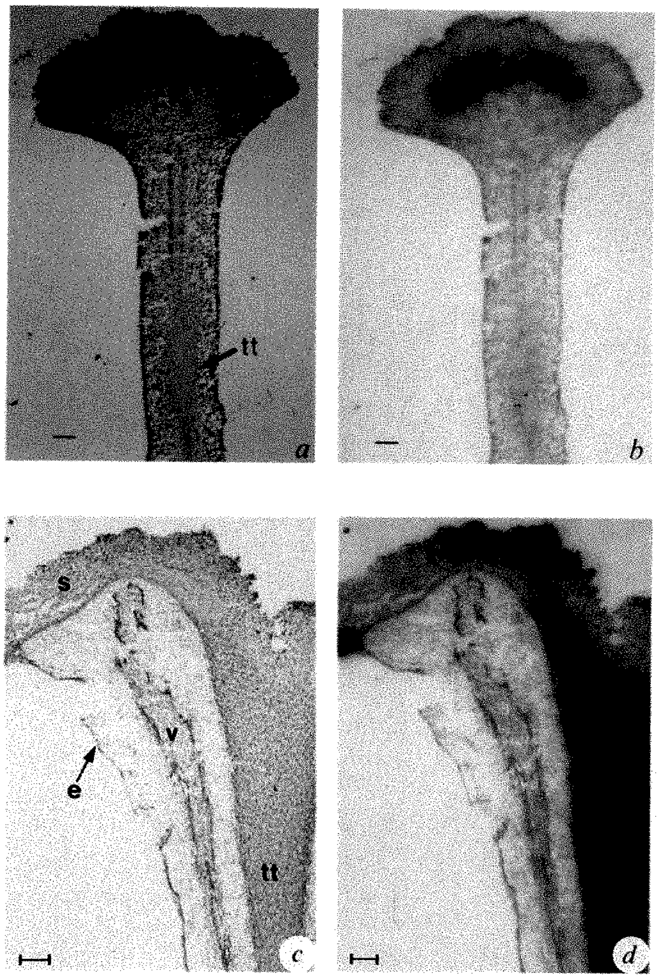

Fig. 3 *a*, *b*, Hybridization of S_2-cDNA to a longitudinal section of an immature pistil of *Nicotiana alata*. *a*, Longitudinal cryostat section through immature pistil; *b*, composite image produced with autoradiograph of same section after hybridization of ^{32}P-labelled S_2-cDNA. Binding is restricted to two sites in the secretory epidermis of the stigma. The transmitting tract (tt) is differentiated but does not bind the S_2-cDNA. Bar, 100 μm. *c*, *d*, Hybridization of S_2-cDNA to a longitudinal section of a mature pistil of *Nicotiana alata*. *c*, Longitudinal cryostat section of upper 2.5 mm of mature pistil corresponding to area 1 in Fig. 1, showing the structural continuity between the secretory tissue of the stigma (s) and the transmitting tissue (tt) of the style; v, vascular bundle. *d*, Composite image produced with autoradiograph of the same section after hybridization of ^{32}P-labelled S_2-cDNA, showing localization of S_2-mRNA restricted to secretory tissue of the stigma and transmitting tract. There is no binding to the outer epidermis (e) of the style, the vascular bundle or the cortical parenchyma. Bar, 100 μm.

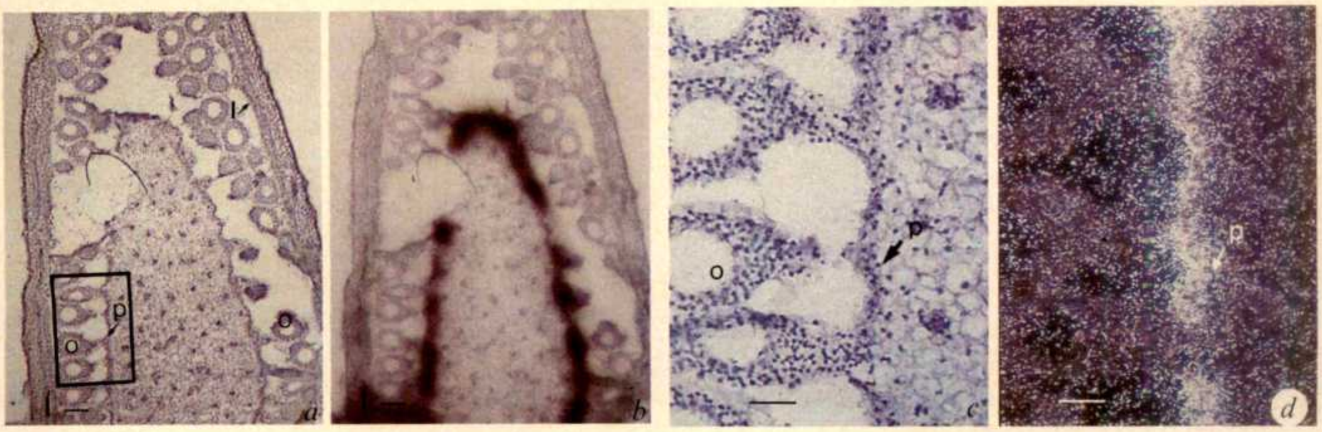

Fig. 4 Hybridization of S_2 cDNA to a longitudinal section of a mature ovary. *a*, Longitudinal cryostat section of mature ovary, corresponding to area 2 of Fig. 1. The epidermis of the placenta (p), the epidermis of the locule (l) and the ovules (o) are indicated. The section is damaged and does not show the complete ovary; this is usual with cryostat sections of organs such as ovary which contain large, fluid-filled cavities. Bar, 100 μm. *b*, Composite image produced with autoradiograph of same section as *a* after hybridization of ^{32}P-labelled S_2 cDNA, showing localization in epidermis of the placenta. *c*, *d*, Section from the boxed area of *a* after hybridization and liquid emulsion autoradiography. *c*, Bright-field micrograph showing four ovules (o) attached to the placenta. The epidermis of the placenta (p) is indicated. Bar, 50 μm. *d*, The same section as *c* photographed with dark-field illumination. The white area over the epidermis of the placenta (p) corresponds to silver particles produced from the action of the bound ^{32}P-cDNA on the photographic emulsion. The use of liquid emulsion enhances the resolution of the technique, enabling localization to particular cell layers.

Methods. Flowers of *Nicotiana alata* of self-incompatibility genotypes S_2S_3 were obtained as described[2]. Pistils were excised and immersed in OCT compound (Lab-Tek Products) in an embedding mould and frozen in Freon cooled with liquid nitrogen. Sections (6–8 μm) were cut on a Reichert Jung cryostat at −12 to −15 °C. The sections were transferred to slides pre-coated with 1% gelatine hardened with 0.25% formaldehyde by pressing the slide lightly against the section. Slides were transferred to a block of dry ice for ⩾30 min to enhance adhesion of section to slide[9]. Control sections were incubated with ribonuclease A (Boeringher-Mannheim; 0.5 mg ml^{-1}, 37 °C, 2 h) and washed. The sections were lightly fixed (2% glutaraldehyde, 0.15 M sodium phosphate buffer, *p*H 7.2, 33% ethylene glycol, 4 °C, 5–10 min), washed briefly in hybridization buffer (5 × Denhardt's, 50 mM sodium-phosphate *p*H 7.0, 600 mM NaCl, 5 mM EDTA, 1 mg ml^{-1} sonicated herring sperm DNA and 50% deionized formamide), first at room temperature and then at 38 °C. Sections were pre-hybridized with hybridization buffer; 30 min, 38 °C and washed sequentially in 1 × SSC and ethanol (AR grade). For hybridization, labelled probe (6 ng DNA, 6×10^5 c.p.m.) was applied in a volume large enough to cover the entire section (usually about 3 μl). The section was covered with a coverslip and a sheet of parafilm, stored in an atmosphere of 50% formamide and incubated for 30 h at 40 °C. Sections were then washed sequentially in 4 × SSC at room temperature, 2 × SSC at room temperature, 1 × SSC for 30 min at 38 °C with gentle agitation and finally in ethanol (AR grade). Slides were exposed directly to X-ray film (Cronex MRF 32, Dupont). The autoradiograph was overlaid on the stained section to give the composite shown. Alternatively, slides were subjected to liquid emulsion autoradiography (Ilford Nuclear Research Emulsion)[9]. Slides were counterstained with toluidine blue (0.025%) to show the tissue structure. Probes of cDNA were labelled to a specific activity of 10^8 c.p.m. g^{-1} using random priming[10]. Control experiments demonstrated that the hybridization observed represents specific binding to RNA. To demonstrate that RNA was preserved, pre-hybridized sections were stained for nucleic acid with acridine orange[11]. There was intense orange staining in all tissue types, but pre-incubation of replicate sections with ribonuclease A completely abolished the orange staining. RNA preservation was also demonstrated using a cDNA probe for ribosomal RNA. This probe, a 2.6-kilobase *Bam*HI fragment purified[12] from a *Bam*HI digestion of the pea ribosomal DNA plasmid pHA2 (from Dr J. Watson, Carnegie Institution, California), hybridized throughout the pistil tissues. As a negative control, we incubated sections with a cDNA encoding the enzyme (1 → 3) × (1 → 4)-β-glucan hydrolase (EC 3.2.1.73) from barley[13]. This enzyme specifically hydrolyses the (1 → 3)(1 → 4)-glucan characteristic of cereals and grasses. Neither the mixed linked glucan nor the enzyme have been found in dicotyledons[14]. There was no binding of the (1 → 3)(1 → 4)-β-glucan hydrolase cDNA to any sections.

tissue expressing the *S*-gene product. We suggest that the low level of expression restricted to the stigma in the immature flower is insufficient to induce pollen-tube arrest.

Hybridization to sections of the mature pistil (Fig. 3) showed that the S_2-gene was expressed throughout the transmitting tract of the style and also in the continuation of this tissue in the stigma. The expression of this gene, initially at a low level in the immature style and then at a high level in the mature style, is consistent with a number of earlier observations of developmentally regulated expression of self-incompatibility associated style proteins[3,5]. There was no hybridization to the outer epidermal or cortical cells of the mature style. The gene is expressed in the ovary of the mature flower but only in the epidermal secretory cells of the placenta (Fig. 4). As these cells are continuous with and have the same origin as those of the transmitting tract, hybridization of the S_2 cDNA is not unexpected. That the hybridization does not extend into the epidermis of the carpel wall (the outer epidermis of the locule) and of the ovary is probably connected to the early, independent development of the placenta in relation to the other carpel tissues[6]. The pattern of S_2-gene expression, first in the immature stigma but not in the placenta or the transmitting tissue of the style, and later in all three, reflects the developmental sequence of the continuous secretory tissue[4]. *S*-gene expression in the mature style coincides precisely with the pathway that compatible pollen tubes take during their growth to the ovary (Figs 1 and 2). *S*-gene expression in the placenta may have a physiological function in arresting growth of any self-pollen tubes which escape the normal inhibition zone. It might also represent an ancestral functional condition in the carpel; the properties of the placental epidermis being transferred, according to Corner's principle[7], to the transmitting tract of the style with the evolution of the terminal stigma. In certain species with hollow styles or without styles, gametophytic self-incompatibility is characterized by pollen-tube inhibition within the ovary. The present observations give a molecular basis for the interpretation[8] of ovarian incompatibility as delayed expression of a normal gametophytically controlled *S*-gene.

This study illustrates the power of hybridization histochemistry in localizing gene expression in plant tissues and cell types; it will be valuable for analysing *S*-gene expression in pollen, which to date has eluded biochemical analysis.

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Note added in proof: Since this paper was submitted we have raised an antibody against a synthetic peptide corresponding to amino acids 45–66[2] of the S_2 glycoprotein. Immunofluorescence microscopy with this antibody has shown that the S_2 mRNA is translated both in the style transmitting tract and the epidermal cells of the placenta of the mature *N. alata* pistil.

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Sequences of *S*-glycoproteins, products of the *Brassica campestris* self-incompatibility locus

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The cruciferous genus *Brassica* has a self-incompatibility system that is under the sporophytic control of a single locus, *S* (ref. 1). Although evidence has accumulated that *S*-locus-specific proteins, termed *S*-glycoproteins[2–4], are strongly associated with the expression of self-incompatibility[4–8], their structures and functions are still unknown. This paper reports the sequences of the *S*-glycoproteins from three homozygotes of *Brassica campestris*. They show extensive homology. The published DNA sequence data for the S_6-glycoprotein of *B. oleracea*[9] can be revised to encode the total sequence of the glycoprotein on the basis of the sequences of *S*-glycoproteins of *B. campestris*. These *S*-glycoproteins have cysteine-rich clusters and 6–7 oligosaccharide chains. There is no homology between the *S*-glycoproteins of *Nicotiana alata*[10] and those of *B. campestris*.

In our previous papers[11,12], we reported the isolation of the *S*-glycoproteins of three homozygotes, S_8S_8, S_9,S_9 and $S_{12}S_{12}$ of *B. campestris* and their amino-terminal sequences. We also reported structures of the major *N*-glycosidic oligosaccharide chains of the total stigma glycoproteins of the homozygous genotype S_8S_8 and presented evidence that these may be identical with the structures of the oligosaccharide chains of the *S*-glycoproteins of *B. campestris*.

Peptide fragments from S_8-glycoprotein were obtained by cyanogen bromide fragmentation and digestion of the isolated glycoprotein with proteolytic enzymes. Sequence analysis of the intact glycoprotein and the peptide fragments revealed five partial sequences (Fig. 1*a*). The relative molecular mass (M_r of the protein part of S_8-glycoprotein was calculated to be $\sim$47,000 (47K) because S_8-glycoprotein exhibited a broad band of M_r 53K on SDS-polyacrylamide gel electrophoresis[11] and should contain four or five *N*-glycosidic carbohydrate chains[12]. Therefore the sequences in Fig. 1*a* should cover $\sim$95% of the whole protein sequence.

The HPLC pattern of lysyl endopeptidase digests of the S_8-glycoprotein (Fig. 1*b*), was quite different from those of S_9- and S_{12}-glycoproteins. The sequencing of these fragment peptides of S_9- and S_{12}-glycoproteins revealed many peptide fragments homologous with those of S_8-glycoprotein (Fig. 1*a*). These results indicate that although displacement of amino acids was observed at many places, these *S*-glycoproteins are similar to each other over the whole region. This is consistent with the hypothesis that these are products of alleles of a single locus. The underlines sequences in fragment C in Fig. 1*a* have parts that differ between the established sequences.

Several X–(a.a.)–Ser/Thr sequences were found, where X is a *N*-glycosylated asparaginyl residue of the type commonly observed in glycoproteins[13] and a.a. is any amino acid. Six such sites were found in the fragments of S_8-glycoprotein. Note that amino acid 35 in fragment C of S_8-glycoprotein, which is probably also a glycosylated asparagine, is replaced by aspartic acid in S_{12}-glycoprotein. The fact that no galactose or arabinose was detected in the acid hydrolysate of the S_8-glycoprotein (data not shown) shows that there may be no *O*-glycosidic saccharide chains in the *S*-glycoproteins of *B. campestris*.

Recently Nasrallah *et al.* reported a complementary DNA sequence encoding part of the S_6-locus-specific glycoprotein (S_6-glycoprotein)[9] in *B. oleracea*, a species very closely related to *B. campestris*. Their proposed nucleotide sequence of S_6-glycoprotein (Fig. 2) is composed of 1,283 base pairs (bp). As there is a nonsense codon (TGA) at base pair positions 370–372 in the reading frame, they concluded that the nucleotides encode 123 amino-acid residues of the carboxy-terminal sequence of S_6-glycoprotein. But a comparison of our peptide sequences of S_8-, S_9- and S_{12}-glycoproteins with their cDNA sequence of S_6-glycoprotein reveals the following. (1) A peptide sequence homologous to our amino-terminal sequences (fragment A) is found at their proposed carboxy-terminal region (from position 40). (2) The sequences homologous with fragments D and E can be observed in the downstream region of the termination codon, from positions 931 and 1,042, respectively, which was previously thought to be an unusual 3′-noncoding region. (3) Fragment C can be arranged on their sequence as a homologous sequence starting from position 292. The homology suddenly breaks after Arg at position 328, but if one base pair could be inserted between 330 and 337, the homology with their sequence would continue.

Immunological analyses suggest that the *S*-glycoproteins of both the two *Brassica* species have a common structure[5]. We have found that the amino-terminal sequence of the S_{39}-glycoprotein of *B. oleracea*[14] is INTLSXTESLXI, which is very similar to the corresponding amino terminus of *B. campestris*. These data strongly suggest that the *S*-glycoproteins of these *Brassica* species are mutually homologous and that the amino-terminal residue of S_6-glycoprotein is isoleucine at the amino acid 14 of the protein sequence deduced from the cDNA. The peptide before the amino terminus of S_6-glycoprotein (dashed line in Fig. 2), with a sequence characteristic of a signal peptide[15] should be a carboxy-terminal part of the signal peptide, because the initiation codon methionine was not present in the cDNA sequenced. Therefore, insertion of one base pair each at positions 333–334, 766–767 and 812–813 is the most plausible revision to resolve the contradiction between our peptide sequences and the cDNA of Nasrallah *et al.* It results in a longer open reading frame encoding 405 amino acids and ending at the TAG codon at base-pair position 1,252. Then all the peptide fragments of S_8-glycoprotein can be aligned homologously with the putative amino-acid sequence of S_6-glycoprotein. The amino-acid sequences of these two *S*-glycoproteins show >80% similarity. The part which varies in S_8- and S_{12}-glycoproteins (underlined in Fig. 1*a*) was greatly different also in S_6-glycoprotein (underlined with a wide bar in Fig. 2). We shall assume that in reading the base pairs at three positions in the published cDNA sequence of the S_6-glycoprotein, a base pair was inadvertently omitted from the sequence, as has occasionally occurred.

We propose the primary structure of S_8-glycoprotein drawn schematically in Fig. 3*a*. The putative amino-acid sequence of S_6-glycoprotein had nine potential glycosylation sites and they may be almost the same as those in S_8-glycoprotein shown in Fig. 2. Six of the nine sites were thought to be glycosylated in the S_8-glycoprotein because no PTH-amino acids were detected

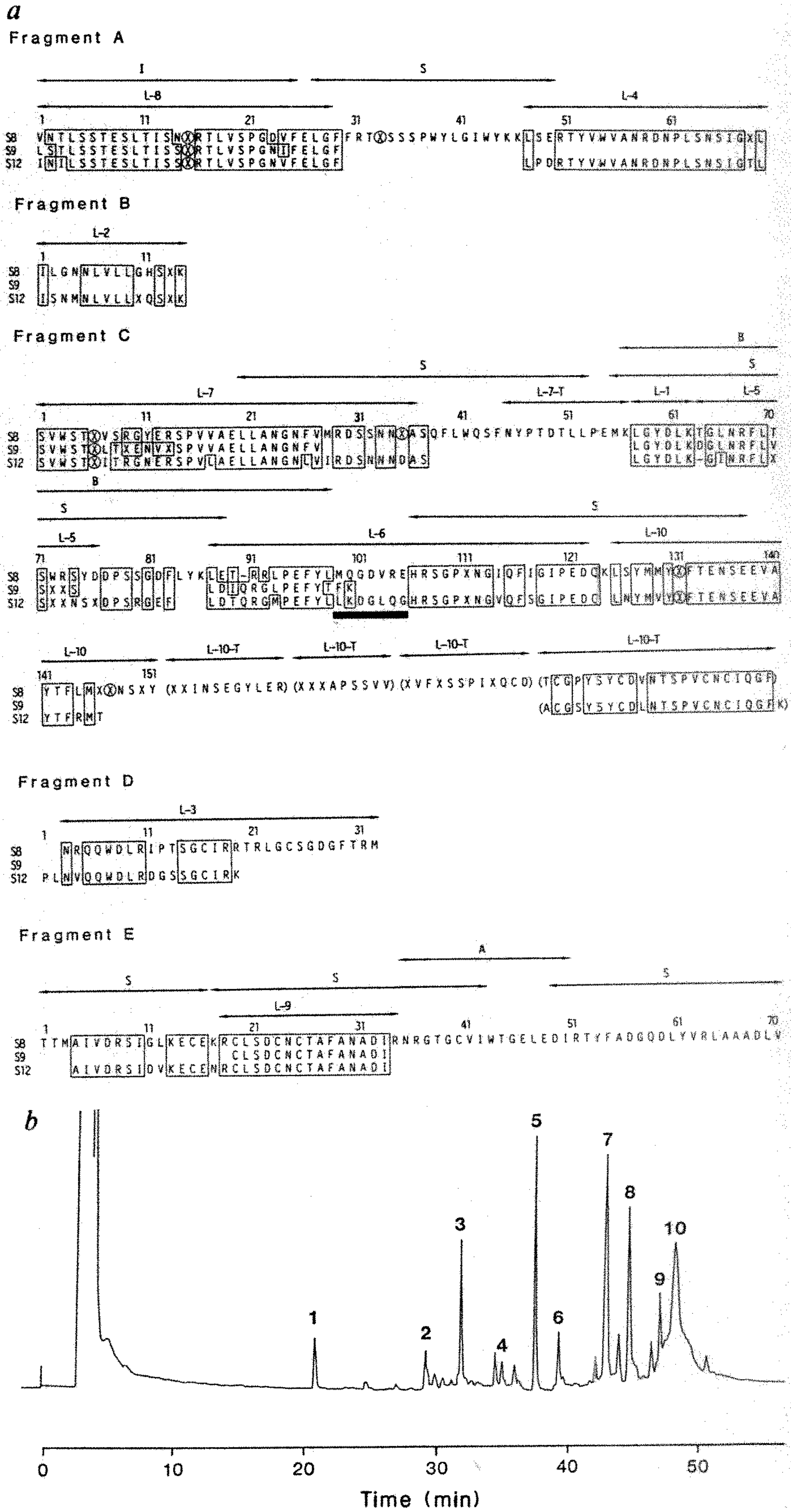

Fig. 1 Determination of amino-acid sequence. *a*, Amino-acid sequences of S_8-, S_9-, S_{12}-glycoproteins as fragments from *B. campestris.* Peptides were obtained by cyanogen bromide fragmentation and digestion of S_8-glycoprotein with proteolytic enzymes. The sequences of the intact glycoprotein and the peptide fragments determined by sequence analysis with gas-phase micro sequencer (Applied Biosystems 470A) are shown as solid lines above the sequence. Letters refer to (I) intact fragments and those digested with (L) lysyl endopeptidase, (T) trypsin, (S) *Staphylococcus aureus* V8 protease, (A) endoproteinase Arg C and (B) cyanogen bromide. The numbers following L are fragment numbers obtained from preparative HPLC (see *b*). The L-7-T and L-10-T sequences are the trypsin digestion fragments of L-7 and L-10 fragments, respectively. All the peptide sequences of S_9- and S_{12}-glycoproteins were obtained from their lysyl endopeptidase peptides and arranged under the homologous sequences of S_8-glycoprotein. In fragment E two V8 protease peptides 1–16 and 17–42 in the S_8-glycoprotein are linked because of a homologous lysyl endopeptidase fragment, 14–33, of the S_{12}-glycoprotein. Each amino acid is designated by one letter (A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr). Unidentified residues are indicated by X, including the glycosylated asparaginyl residue; circled X is a potential glycosylation site. Gaps indicated by hyphens are introduced to optimize alignment of similar regions. Residues the same in more than two *S*-glycoproteins are boxed. *b*, HPLC chromatogram of lysl endopeptidase fragments of S_8-glycoprotein after modification of disulphide bonds with iodoacetamide.

Methods. The *S*-glycoproteins were purified from stigmas of S_8S_8, S_9S_9 and $S_{12}S_{12}$ homozygotes of *B. campestris* by a combination of Sephadex G-100 gel filtration, concanavalin A/Sepharose affinity chromatography, ion-exchange HPLC and reverse-phase HPLC[11]. In each step of the purification, the *S*-glycoproteins were followed by monitoring the appropriate band on polyacrylamide isoelectric focusing gels (isoelectric points for S_8, S_9 and S_{12} were 8.4, 6.9 and 5.7, respectively). For proteolytic digestion, the cysteinyl residues were first reduced and alkylated. *S*-glycoprotein (40–80 μg) was dissolved in 30 μl of deoxygenated 0.5 M Tris HCl/6M urea (*p*H 8.5). Dithiothreitol was added to a final concentration of 4–6 mM and incubated at 45 °C for 1 h. Then a solution (10 μl) of either 40 mM iodoacetamide or vinylpyridine was added and the incubation was continued in the dark at room temperature for 20 min. The alkylation was terminated by adding a solution (10 μl) of 40 mM cysteine hydrochloride. The reaction mixture was diluted with water to 400 μl and subjected to the following proteolytic digestion without further purification. The enzymes, their concentrations and other conditions used were as follows. Lysyl endopeptidase (Wakojunyaku): 1:20 molar ratio of enzyme to substrate, 37 °C, 24 h; L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin (Sigma): 1:20 (w/w), 37 °C, 3 h; *Staph. aureus* V8 protease (Miles): 1:15 (w/w), 37 °C, 20 h; endoproteinase Arg C (Sigma): 1:10 (w/w), 37 °C, 3 h. In each case the enzymatic digestion was stopped by adding 1–2 μl of formic acid. A cysteine-rich fragment obtained by treating with lysyl endopeptidase was further cleaved by trypsin yielding several small fragments. For cyanogen bromide digestion, S_8-glycoprotein (67 μg) was first dissolved in 50 μl of 70% formic acid. A 100-fold excess of cyanogen bromide was added and the incubation was continued overnight in the dark at room temperature. The reaction mixture was then diluted ten times with water and lyophilized. All digestion fragments were fractionated on a Hi-Pore RP-304 column (4.6 × 250 mm, Bio-Rad) with a linear gradient of 0–60% acetonitrile in 0.1% trifluoroacetic acid (60 min). The peptide fragments were detected by UV absorbance at 220 nm. Amino-acid sequencing was done on a gas-phase microsequencer (Applied Biosystems 470A). The PTH-amino acid obtained from each sequencing cycle was identified by an on-line PTH-amino acid analyser (Applied Biosystems 120A). Average repetitive yield in Edman degradation was 92%.

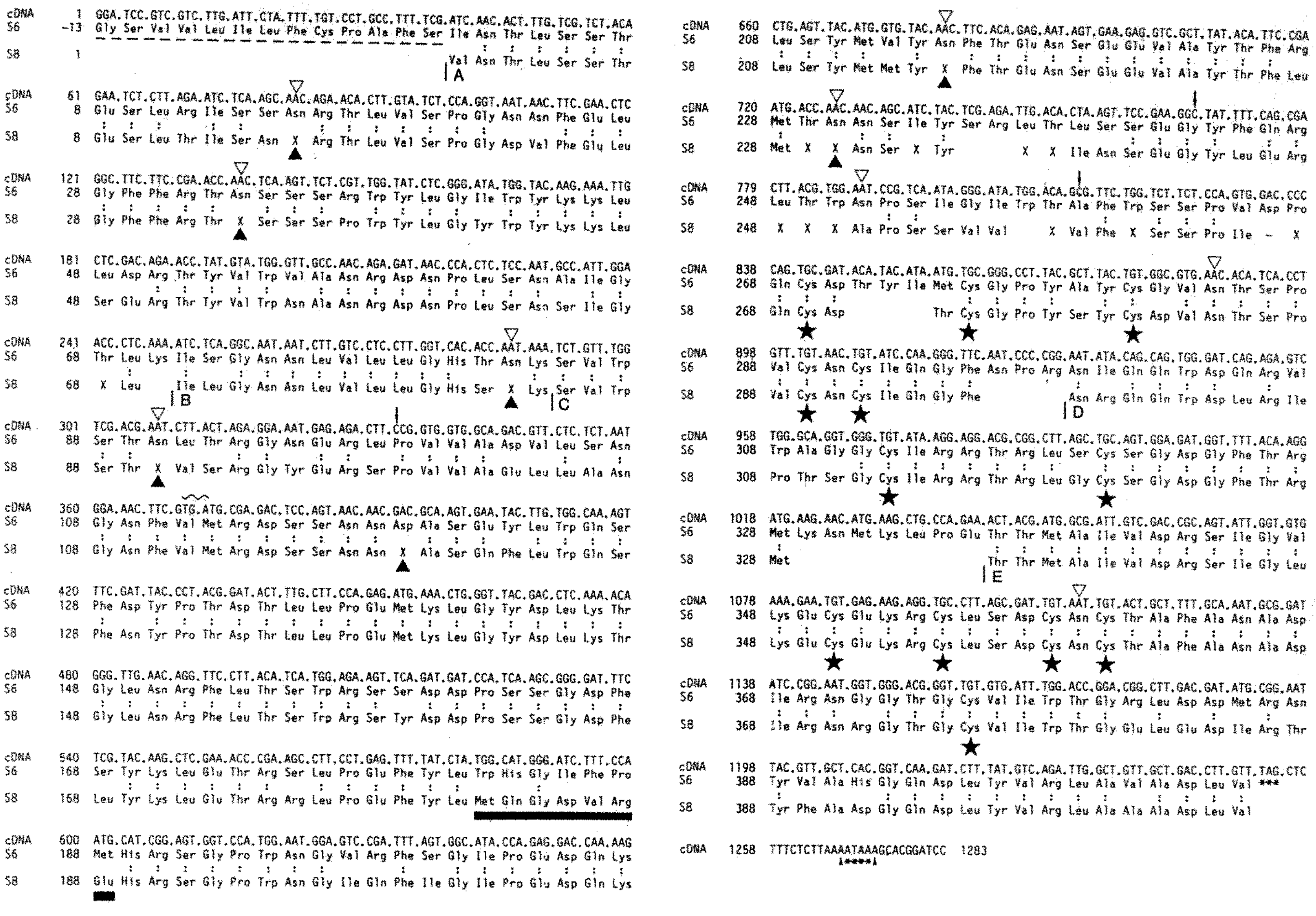
cDNA 1 GGA.TCC.GTC.GTC.TTG.ATT.CTA.TTT.TGT.CCT.GCC.TTT.TCG.ATC.AAC.ACT.TTG.TCG.TCT.ACA
S6 -13 Gly Ser Val Val Leu Ile Leu Phe Cys Pro Ala Phe Ser Ile Asn Thr Leu Ser Ser Thr
S8 1 Val Asn Thr Leu Ser Ser Thr
A
cDNA 61 GAA.TCT.CTT.AGA.ATC.TCA.AGC.AAC.AGA.ACA.CTT.GTA.TCT.CCA.GGT.AAT.AAC.TTC.GAA.CTC
S6 8 Glu Ser Leu Arg Ile Ser Ser Asn Arg Thr Leu Val Ser Pro Gly Asn Asn Phe Glu Leu
S8 8 Glu Ser Leu Thr Ile Ser Asn X Arg Thr Leu Val Ser Pro Gly Asp Val Phe Glu Leu
cDNA 121 GGC.TTC.TTC.CGA.ACC.AAC.TCA.AGT.TCT.CGT.TGG.TAT.CTC.GGG.ATA.TGG.TAC.AAG.AAA.TTG
S6 28 Gly Phe Phe Arg Thr Asn Ser Ser Ser Arg Trp Tyr Leu Gly Ile Trp Tyr Lys Lys Leu
S8 28 Gly Phe Phe Arg Thr X Ser Ser Ser Pro Trp Tyr Leu Gly Tyr Trp Tyr Lys Lys Leu
cDNA 181 CTC.GAC.AGA.ACC.TAT.GTA.TGG.GTT.GCC.AAC.AGA.GAT.AAC.CCA.CTC.TCC.AAT.GCC.ATT.GGA
S6 48 Leu Asp Arg Thr Tyr Val Trp Val Ala Asn Arg Asp Asn Pro Leu Ser Asn Ala Ile Gly
S8 48 Ser Glu Arg Thr Tyr Val Trp Asn Ala Asn Arg Asp Asn Pro Leu Ser Asn Ser Ile Gly
cDNA 241 ACC.CTC.AAA.ATC.TCA.GGC.AAT.AAT.CTT.GTC.CTC.CTT.GGT.CAC.ACC.AAT.AAA.TCT.GTT.TGG
S6 68 Thr Leu Lys Ile Ser Gly Asn Asn Leu Val Leu Leu Gly His Thr Asn Lys Ser Val Trp
S8 68 X Leu Ile Leu Gly Asn Asn Leu Val Leu Leu Gly His Ser X Lys Ser Val Trp
B
C
cDNA 301 TCG.ACG.AAT.CTT.ACT.AGA.GGA.AAT.GAG.AGA.CTT.CCG.GTG.GTG.GCA.GAC.GTT.CTC.TCT.AAT
S6 88 Ser Thr Asn Leu Thr Arg Gly Asn Glu Arg Leu Pro Val Val Ala Asp Val Leu Ser Asn
S8 88 Ser Thr X Val Ser Arg Gly Tyr Glu Arg Ser Pro Val Val Ala Glu Leu Leu Ala Asn
cDNA 360 GGA.AAC.TTC.GTG.ATG.CGA.GAC.TCC.AGT.AAC.AAC.GAC.GCA.AGT.GAA.TAC.TTG.TGG.CAA.AGT
S6 108 Gly Asn Phe Val Met Arg Asp Ser Ser Asn Asn Asp Ala Ser Glu Tyr Leu Trp Gln Ser
S8 108 Gly Asn Phe Val Met Arg Asp Ser Ser Asn Asn X Ala Ser Gln Phe Leu Trp Gln Ser
cDNA 420 TTC.GAT.TAC.CCT.ACG.GAT.ACT.TTG.CTT.CCA.GAG.ATG.AAA.CTG.GGT.TAC.GAC.CTC.AAA.ACA
S6 128 Phe Asp Tyr Pro Thr Asp Thr Leu Leu Pro Glu Met Lys Leu Gly Tyr Asp Leu Lys Thr
S8 128 Phe Asn Tyr Pro Thr Asp Thr Leu Leu Pro Glu Met Lys Leu Gly Tyr Asp Leu Lys Thr
cDNA 480 GGG.TTG.AAC.AGG.TTC.CTT.ACA.TCA.TGG.AGA.AGT.TCA.GAT.GAT.CCA.TCA.AGC.GGG.GAT.TTC
S6 148 Gly Leu Asn Arg Phe Leu Thr Ser Trp Arg Ser Ser Asp Asp Pro Ser Ser Gly Asp Phe
S8 148 Gly Leu Asn Arg Phe Leu Thr Ser Trp Arg Ser Tyr Asp Asp Pro Ser Ser Gly Asp Phe
cDNA 540 TCG.TAC.AAG.CTC.GAA.ACC.CGA.AGC.CTT.CCT.GAG.TTT.TAT.CTA.TGG.CAT.GGG.ATC.TTT.CCA
S6 168 Ser Tyr Lys Leu Glu Thr Arg Ser Leu Pro Glu Phe Tyr Leu Trp His Gly Ile Phe Pro
S8 168 Leu Tyr Lys Leu Glu Thr Arg Arg Leu Pro Glu Phe Tyr Leu Met Gln Gly Asp Val Arg
cDNA 600 ATG.CAT.CGG.AGT.GGT.CCA.TGG.AAT.GGA.GTC.CGA.TTT.AGT.GGC.ATA.CCA.GAG.GAC.CAA.AAG
S6 188 Met His Arg Ser Gly Pro Trp Asn Gly Val Arg Phe Ser Gly Ile Pro Glu Asp Gln Lys
S8 188 Glu His Arg Ser Gly Pro Trp Asn Gly Ile Gln Phe Ile Gly Ile Pro Glu Asp Gln Lys
cDNA 660 CTG.AGT.TAC.ATG.GTG.TAC.AAC.TTC.ACA.GAG.AAT.AGT.GAA.GAG.GTC.GCT.TAT.ACA.TTC.CGA
S6 208 Leu Ser Tyr Met Val Tyr Asn Phe Thr Glu Asn Ser Glu Glu Val Ala Tyr Thr Phe Arg
S8 208 Leu Ser Tyr Met Met Tyr X Phe Thr Glu Asn Ser Glu Glu Val Ala Tyr Thr Phe Leu
cDNA 720 ATG.ACC.AAC.AAC.AGC.ATC.TAC.TCG.AGA.TTG.ACA.CTA.AGT.TCC.GAA.GGC.TAT.TTT.CAG.CGA
S6 228 Met Thr Asn Asn Ser Ile Tyr Ser Arg Leu Thr Leu Ser Ser Glu Gly Tyr Phe Gln Arg
S8 228 Met X X Asn Ser X Tyr X X Ile Asn Ser Glu Gly Tyr Leu Glu Arg
cDNA 779 CTT.ACG.TGG.AAT.CCG.TCA.ATA.GGG.ATA.TGG.ACA.GCG.TTC.TGG.TCT.TCT.CCA.GTG.GAC.CCC
S6 248 Leu Thr Trp Asn Pro Ser Ile Gly Ile Trp Thr Ala Phe Trp Ser Ser Pro Val Asp Pro
S8 248 X X X Ala Pro Ser Ser Val Val X Val Phe X Ser Ser Pro Ile - X
cDNA 838 CAG.TGC.GAT.ACA.TAC.ATA.ATG.TGC.GGG.CCT.TAC.GCT.TAC.TGT.GGC.GTG.AAC.ACA.TCA.CCT
S6 268 Gln Cys Asp Thr Tyr Ile Met Cys Gly Pro Tyr Ala Tyr Cys Gly Val Asn Thr Ser Pro
S8 268 Gln Cys Asp Thr Cys Gly Pro Tyr Ser Tyr Cys Asp Val Asn Thr Ser Pro
cDNA 898 GTT.TGT.AAC.TGT.ATC.CAA.GGG.TTC.AAT.CCC.CGG.AAT.ATA.CAG.CAG.TGG.GAT.CAG.AGA.GTC
S6 288 Val Cys Asn Cys Ile Gln Gly Phe Asn Pro Arg Asn Ile Gln Gln Trp Asp Gln Arg Val
S8 288 Val Cys Asn Cys Ile Gln Gly Phe Asn Arg Gln Gln Trp Asp Leu Arg Ile
D
cDNA 958 TGG.GCA.GGT.GGG.TGT.ATA.AGG.AGG.ACG.CGG.CTT.AGC.TGC.AGT.GGA.GAT.GGT.TTT.ACA.AGG
S6 308 Trp Ala Gly Gly Cys Ile Arg Arg Thr Arg Leu Ser Cys Ser Gly Asp Gly Phe Thr Arg
S8 308 Pro Thr Ser Gly Cys Ile Arg Arg Thr Arg Leu Gly Cys Ser Gly Asp Gly Phe Thr Arg
cDNA 1018 ATG.AAG.AAC.ATG.AAG.CTG.CCA.GAA.ACT.ACG.ATG.GCG.ATT.GTC.GAC.CGC.AGT.ATT.GGT.GTG
S6 328 Met Lys Asn Met Lys Leu Pro Glu Thr Thr Met Ala Ile Val Asp Arg Ser Ile Gly Val
S8 328 Met Thr Thr Met Ala Ile Val Asp Arg Ser Ile Gly Leu
E
cDNA 1078 AAA.GAA.TGT.GAG.AAG.AGG.TGC.CTT.AGC.GAT.TGT.AAT.TGT.ACT.GCT.TTT.GCA.AAT.GCG.GAT
S6 348 Lys Glu Cys Glu Lys Arg Cys Leu Ser Asp Cys Asn Cys Thr Ala Phe Ala Asn Ala Asp
S8 348 Lys Glu Cys Glu Lys Arg Cys Leu Ser Asp Cys Asn Cys Thr Ala Phe Ala Asn Ala Asp
cDNA 1138 ATC.CGG.AAT.GGT.GGG.ACG.GGT.TGT.GTG.ATT.TGG.ACC.GGA.CGG.CTT.GAC.GAT.ATG.CGG.AAT
S6 368 Ile Arg Asn Gly Gly Thr Gly Cys Val Ile Trp Thr Gly Arg Leu Asp Asp Met Arg Asn
S8 368 Ile Arg Asn Arg Gly Thr Gly Cys Val Ile Trp Thr Gly Glu Leu Glu Asp Ile Arg Thr
cDNA 1198 TAC.GTT.GCT.CAC.GGT.CAA.GAT.CTT.TAT.GTC.AGA.TTG.GCT.GTT.GCT.GAC.CTT.GTT.TAG.CTC
S6 388 Tyr Val Ala His Gly Gln Asp Leu Tyr Val Arg Leu Ala Val Ala Asp Leu Val ***
S8 388 Tyr Phe Ala Asp Gly Gln Asp Leu Tyr Val Arg Leu Ala Ala Ala Asp Leu Val
cDNA 1258 TTTCTCTTAAAATAAAGCACGGATCC 1283

Fig. 2 Revised cDNA sequence and the deduced amino-acid sequence of S_6-glycoprotein, and the alignment of the predicted sequences with S-glycoprotein amino-acid sequence. The cDNA and amino acids of two proteins are numbered on the left. Top sequence, cDNA: the revised cDNA sequence of the S_6-glycoprotein. Position numbers are unchanged from the published data[9], and the inserted bases (tentatively C) are marked with vertical arrows. The codon with the wavy line over it indicates the TGA codon proposed as the termination codon in the published data[9]. Arrows and asterisks below nucleotide sequence, potential polyadenylation signal site[19]. S6: the amino-acid sequence of the S_6-glycoprotein deduced from the above cDNA. Dashed line, proposed part of the signal peptide. The termination codon of the revised cDNA sequence is marked with asterisks in this line. Asn residues marked with open triangles are the potential glycosylation sites deduced from the cDNA sequence of S_6-glycoprotein. S8: determined amino-acid sequence of S_8-glycoprotein. Bold-type capitals under the sequences show the peptide fragments in Fig. 1*a*. X, unidentified amino acid; X marked with closed triangle, potential glycosylation sites deduced from amino-acid sequencing. Identities in the sequences between S_6- and S_8-glycoproteins are marked with two dots between the two lines. Wide bar, variable region which differs between the S-glycoproteins of *B. campestris*, and this part was also different in the S_6-glycoprotein. Twelve cysteine residues are marked with large stars.

in these cycles. Replacements of glycosylated asparagine were observed at amino acids 119 and 251. The estimated M_r of S_8-glycoprotein (53K) corresponds to the sum of 405 amino acids (46K) and 7 oligosaccharide chains.

Oligosaccharides A and B were the main components of N-glycosidic carbohydrate chains in S-glycoprotein (Fig. 3*b*). Oligosaccharide A (M_r 1,391) was predominant and may be the mature form.

The twelve cysteine residues located at the carboxy-terminal end (Fig. 3*a*) should form six disulphide bonds, so this region would form a rigid cluster. In this region, two potential glycosylation sites at amino acids 284 and 359 in S_6-glycoprotein may not be glycosylated as in S_8-glycoprotein. The hydrophilicity index calculated by the method of Hopp and Woods[16] for S_6- and S_8-glycoproteins showed a similar pattern and no highly hydrophobic zone indicating a transmembrane sequence was observed.

Amino-acid sequence similarity has been reported among S-glycoproteins of the gametophytic self-incompatibility systems of *Nicotiana alata* (S_2-glycoprotein) and of *Lycopersicon pervianum*[10] (S_1- and S_3-glycoproteins). The homology reported here is among S-glycoproteins of *Brassica* species, which have sporophytic incompatibility systems. The gametophytic system is generally considered to be the ancestral form of self-incompatibility in flowering plants, with the sporophytic system being derived from it[1]. So homology might be anticipated between the S-glycoproteins of *N. alata* and *Brassica* species. But between two S-glycoproteins of the sporophytic and gametophytic self-incompatibility systems, no amino-acid sequence homology is observed.

A search for peptides homologous to S-glycoproteins of *Brassica* species in a protein database by Gotoh's method[17] indicated that the sequence of the cysteine-rich part has some homology with a family of epidermal growth factor[18]. Whether this is just accidental or has some meaning is not clear.

We thank Dr O. Gotoh of Saitama Cancer Center Research Institute for the use of his system for searching a protein database and to Dr H. Ozawa of the Computer Center of the University

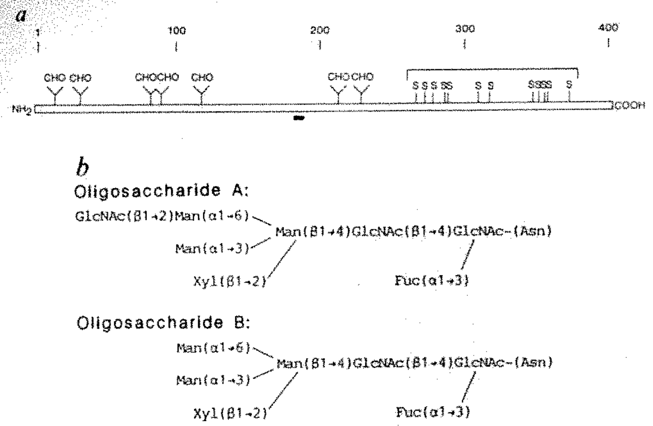

Fig. 3 Schematic drawing of *S*-glycoproteins. *a*, Schematic drawing of the S_8-glycoprotein of *B. campestris.* CHO, carbohydrate chain; S, cysteinyl residue. Heavy bar, zone variable among *Brassica* species. *b*, Structures of oligosaccharides A and B in *S*-glycoproteins of *B. campestris*, oligosaccharide A being predominant. The identity of the major oligosaccharide chains of total stigma proteins with those of *S*-glycoprotein was confirmed by the sequential digestion experiment of the isolated pyridylamino-derivatized saccharide chains from *S*-glycoproteins with various exoglycosidases in comparison with those from the total stigma glycoproteins and from bromelain. The details of the structural elucidation of the saccharide chains of *S*-glycoproteins of *B. campestris* will be published elsewhere (in preparation).

of Tokyo for permitting us to use his database file. We also thank Professor T. Blundell of University of London for his critical reading of the manuscript. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (Nos 60125004 and 61117002).

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Erratum

Glucocorticoid receptor mutants that are constitutive activators of transcriptional enhancement

Paul J. Godowski, Sandro Rusconi, Roger Miesfeld & Keith R. Yamamoto

Nature **325**, 365-368 (1987).

In this letter Figs 3 and 4 were printed incorrectly, without the arrows referred to in the legends. The figures appear correctly below.

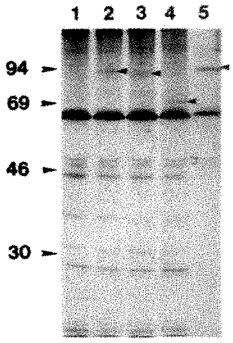

Fig. 4

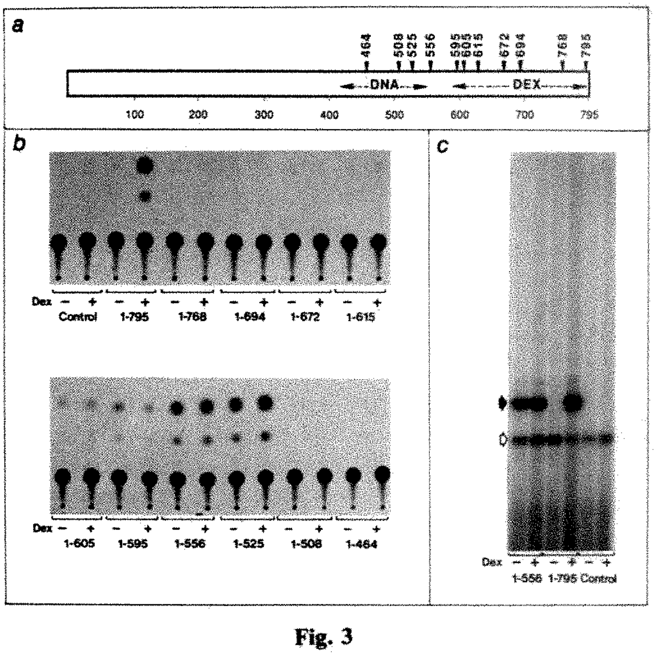

Fig. 3

MATTERS ARISING

Hyperacuity and the visual cortex

Recently Swindale and Cynader[1] demonstrated experimentally that single neurons in cat visual cortex show a form of hyperacuity. They drew several comparisons with our own measurements of hyperacuity in the monkey visual cortex and with our theoretical analysis[2]. They (and Martin[3]) support the presumption that the limiting factors in hyperacuity, in particular Vernier acuity, are purely cortical, in contrast to the limiting factors in resolution acuity, which are thought to be essentially retinal.

Their results showed that a moving Vernier target caused maximum stimulation of a cortical cell when the bars of the target were exactly collinear. Progressively weaker responses were obtained when the bars were offset in a direction orthogonal to their long axes. Significant decrements in response occurred with offsets smaller than the overall size of the cortical cell's receptive field. They argued that this demonstrated a hyperacuity for relative position of the two bars and that this phenomenon was uniquely cortical.

Suppose the moving Vernier target were to stimulate a very simple model receptive field, consisting of a single excitatory region and simple temporal integrator. Results very similar to those of Swindale and Cynader would be obtained; this would be attributable to the fact that only the collinear version of the Vernier target provides optimal, synchronous stimulation of the receptive field in space and time. A tuning curve for Vernier offset could also be obtained by flashing the targets on the receptive field. It seems premature to claim that such results in cortical cells demonstrate a specific sensitivity for relative position when a simpler explanation based on sensitivity for absolute position is possible.

In the case of cat retinal ganglion cells, experiments have revealed thresholds as low as 1 arc min for simple positional sensitivity and models based on linear summation account for these results[4-6]. For monkey cortical cells our own measurements and theoretical predictions[2] showed positional thresholds as low as 10-20 arc s. With the addition of a temporal component[5], these models also predict the kind of results obtained by Swindale and Cynader in cortical cells.

Indeed, all the current results for cortical cells in hyperacuity tasks are readily predicted from similar linear models of receptive field organization. There is good evidence that linear models can be applied to the spatial summation properties of cortical simple cells and to the linearly-summating subunits of complex cells[7-9]. If a specific nonlinear mechanism for extracting relative position were to be demonstrated in cortical cells this would be highly significant, but present evidence indicates that cortical cells have thresholds in the hyperacuity range by virtue of essentially the same type of mechanism that is present in retinal ganglion cells. This similarity is reinforced by the recognition that the firing of a striate cortical cell is ambiguous in much the same way that the firing of a retinal ganglion cell is ambiguous. Changes of contrast, position, spatial frequency, orientation and direction of drift are all effective in altering the firing rate of cortical neurons.

For these reasons, it is also mistaken to conclude that the existence of cortical neurons with positional sensitivity in the hyperacuity range is an argument against the existence of a fine-grain spatial reconstruction[10]. Strictly speaking, current neurophysiological data do not address this question directly. Psychophysics has pointed to the importance of interpolation over a spatially-sampled luminance profile and to the necessity of constructing an exact signal for spatial location, but it is an open question whether this is accomplished by the construction of a fine-grain spatial map or by other means.

The most interesting feature of comparing the hyperacuity performance of retinal and cortical neurons is that the cortical transformation of information preserves very well the accuracy of the positional signals supplied by the retina, even though cortical cells acquire new forms of selectivity, such as orientation and binocularity. The existence of single neurons at several levels in the visual system with thresholds close to those of a psychophysical observer strikingly demonstrates the precision of organization of the early visual pathways and the high quality of the 'components' used for visual computation.

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SWINDALE AND CYNADER REPLY—It is obvious that the information required to make hyperacuity judgements must be present in the signals from the retina. As such judgements can be made for spots of light that stimulate only small numbers of retinal ganglion cells[1], it is not surprising that absolute positional sensitivity in retinal neurons can be as good as the sensitivity to Vernier offset demonstrated behaviourly. The fact that calculated retinal positional sensitivities[2,3] may be even higher than those needed to account for behavioural hyperacuity implies, as Martin suggested[4], that the limiting factors in hyperacuity are cortical and not retinal in origin. Thus it makes sense to study hyperacuity in the cortex and not, as Parker and Hawken would have us do, in the retina.

Although retinal signals contain the information about absolute position required for hyperacuity judgements, such judgements involve comparisons of the positions of features that are further apart than the sizes of ganglion cell receptive fields[5]. Furthermore, changes in the retinal position of the stimulus (caused for example by eye movements) during or between successive presentations, do not degrade acuity[5]. For both these reasons it is unlikely that a signal from the type of retinal detector hypothesised by Parker and Hawken will, on its own, be much use as a basis for a hyperacuity discrimination.

The claim that our results are "readily predicted" from linear models of receptive field organization is not supported. We know of no theoretical predictions of the acuity of retinal or cortical neurons for moving Vernier stimuli. Our preliminary modelling indicates that linear summation within a simple cell's receptive field would produce tuning curves flatter than those observed, and the same is almost certain to be true for retinal cells. There is however experimental evidence for non-linearities of integration along the receptive field axis of simple cells[6-8] which could increase the Vernier sensitivity of an otherwise linear cell.

It is true that the firing rate of a cortical cell is an ambiguous signal but it is certainly less ambiguous than the firing of a retinal cell, by virtue of the cortical cell's feature selectivity. In any case, these ambiguities may not pose as serious a problem for relating cortical neuronal responses to psychophysics as one might suppose. In psychophysical experiments, potentially confounding variables such as the brightness, contrast, length and velocity of the bars in a Vernier target are normally kept constant, and it is possible that random variations in these parameters would indeed degrade performance. If such variations were present it might still be possible to disambiguate a response by combining signals from cells (for example, by subtracting the signals from cells with the same contrast sensitivity, but different Vernier sensitivities).

There are other reasons besides our experimental results (or our interpretation of them) for supposing that a fine-grain representation of receptive field position is not a necessary prerequisite for explaining hyperacuity. One is that the receptive field sizes of the cells involved in such a representation will be no smaller than those of the more coarsely spaced fields of the cells that form the input. Their positional sensitivities will be no greater than that of their inputs, and it is not clear how this extra stage would help the subsequent extraction of information about relative position. The other evidence is the lack of a neuronal substrate for a fine grain representation in the cat: there is no evidence for a layer of closely packed cells with small non-oriented receptive fields as there is in the monkey. Nevertheless, cats have hyperacuity.

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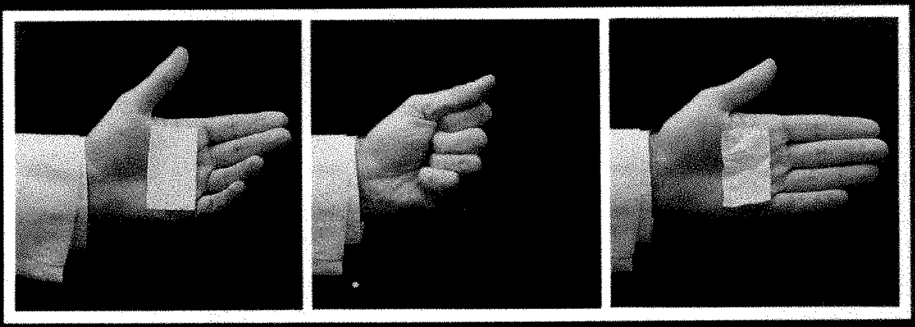

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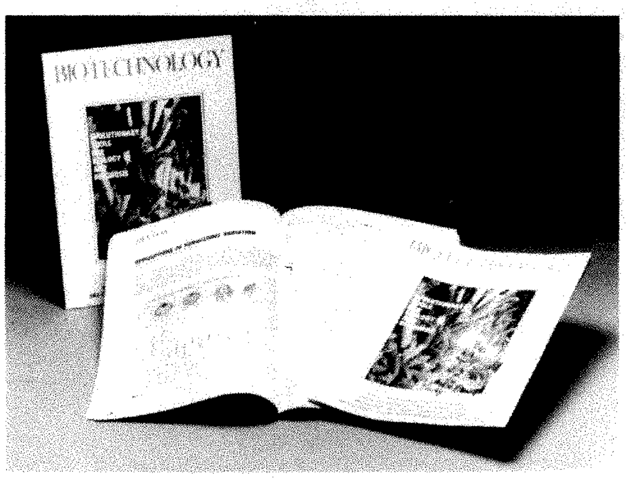

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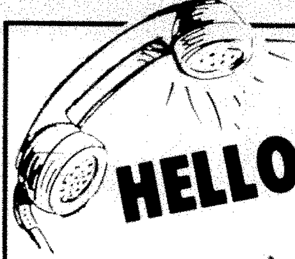

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Tunnelling reveals forbidden transitions

K.W. Hipps

Inelastic electron tunnelling spectroscopy provides unique information about vibrational and electronic transitions that are forbidden to photon-induced processes.

INELASTIC electron tunnelling spectroscopy (IETS) was first developed by Lambe and Jaklevic[1] in 1966. Since that time the technique has evolved and applications now range from the study of biochemicals to transition metal complexes. Several reviews and texts are available[2-4]. IETS is a non-optical method for measuring vibrational and electronic spectra. It is a very sensitive technique, providing the spectrum of a sample containing less than 10^{13} molecules. It has been heavily used for the study of chemisorbed species, as a molecular spectroscopy complementary to infrared (IR) and Raman, and for the observation of transitions which are 'silent' (forbidden) in photon spectroscopy[1-9]. The most unusual feature of the technique is that the sample of interest must be incorporated into a metal–insulator–metal thin film device called a tunnel diode. Thus, sample preparation is more of an art than with IR and Raman spectroscopy. The following discussion describes the use of IETS for the observation of optically forbidden transitions.

More on the method

If two sheets of metal are separated by a thick (>50 nm) insulator, a capacitor results, and the application of a steady potential difference between the plates produces no steady current flow. As the thickness of the insulator is reduced below about 5 nm, however, current does flow and the device has a measurable conductance. This current is due to a quantum mechanical phenomenon known as tunnelling. The electron transmission through the insulator is such that the electron's total energy is less than its potential energy in the insulator (barrier) region. Thus, the electrons seem to be 'tunnelling' through the potential barrier. If the electron moves from the first metal to the second without loss of total energy, the process is termed elastic tunnelling. This is the primary conduction mechanism for tunnel diodes. An inelastic process may also occur. In this case, a portion of the electron's translation energy is lost to the barrier region in the form of vibrational or electronic excitation. It is this inelastic tunnelling process which provides the physical basis for IETS.

Taking I as the current flowing in the junction due to applied (bias) voltage V, a plot of (d^2I/dV^2) versus V yields a tunnelling spectrum[1,2]. Peaks occur at energies (1 volt = 8.066 cm^{-1}) corresponding to electronic or vibrational transitions. The band widths depend strongly on the temperature, and are typically about 15 cm^{-1}, at 4.2 K. Most tunnelling spectra, therefore, are taken at, or below, liquid helium temperature. The intensities of the observed spectra depend on electron–molecule interactions and are not well understood. It is known empirically that Raman active, electric and magnetic dipole active, and optically forbidden transitions may be observed. Transitions having $\Delta S = \pm 1$ and 0 are also observed.

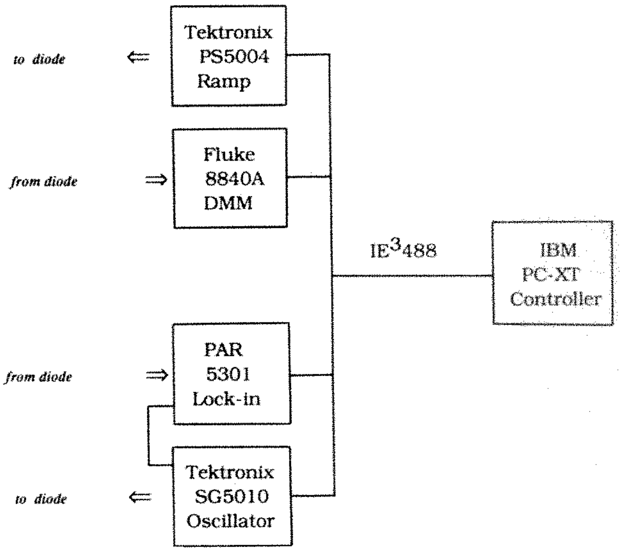

Fig. 1 Schematic drawing of a digital tunnelling spectrometer.

At present, no tunnelling spectrometer is commercially available. There are however, a number of different designs described in the literature[1-5]. The most modern and easily assembled is an all digital spectrometer utilizing an IEEE-488 interface and an IBM-PC-XT controller[5]. A schematic of this spectrometer is shown in Fig 1. In addition to the spectrometer, a high vacuum deposition system is also required to prepare the tunnel diode. Most are prepared in the following manner. A metal film ($\approx$ 100 nm thick × 1 mm × 20 mm) is first deposited on a smooth substrate. An insulating material is then grown or deposited on the metal to form a barrier roughly 1.5 nm thick. The species of interest is placed on the insulator to provide a coverage of about one monolayer. This latter step may be done either from the gas phase or from solution. The second metal electrode is then deposited to complete the tunnel junction. Electrical leads are attached to the metal electrodes, the junction is cooled to 4 K or below, and the spectrum is measured.

Forbidden transitions

In the case of molecular or ionic species having symmetry C_{3v} or higher, there are generally one or more vibrational modes which are not directly observable by either IR or Raman spectroscopy. The number of parameters required to specify the quadratic potential function for atomic motion greatly exceeds the number of observed vibrations in these systems. Thus, the identification of these missing frequencies can significantly improve our understanding of intramolecular forces. Tunnelling spectroscopy can be used to directly observe many of these 'silent' modes.

Figure 2 shows the low energy portion of the tunnelling spectrum of the octahedral symmetry ferrocyanide ion[9]. Also shown is the IR transmission spectrum of the potassium salt of the same ion. The IET spectrum is much richer in strong bands and has similar band widths. Of special interest are the bands at 354 and 474 cm^{-1}. These are due to fundamental motions of the ion which are not observable in either IR or Raman spectroscopy. Another example is provided by the D_{3h} symmetry tricyanomethanide ion $[C(CN)_3^{-1}]$[8]. In this case there is a single in-plane motion which is silent in both IR and Raman. It appears as a fairly intense band in IETS at 402 cm^{-1}.

IETS may also be used to study electronic transitions occurring in the spectral region below about 2.5 eV (20,000 cm^{-1})[2,6]. As in the case of vibrational transitions, both optically allowed and forbidden electronic transitions may be observed.

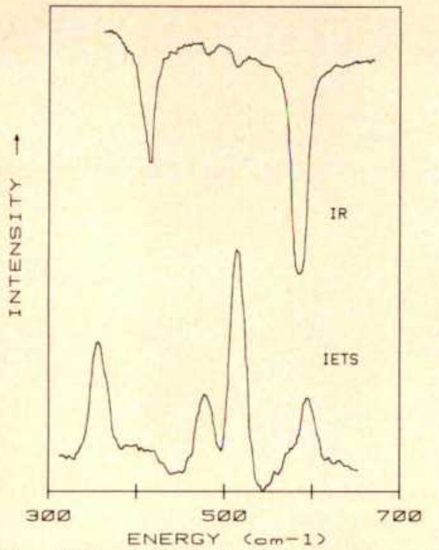

Fig. 2 Tunnelling and infrared spectra of $K_4Fe(CN)_6$.

Through IETS it is now possible to begin probing electronic states which have been theoretically predicted but never observed. This should lead to a much better understanding of phenomena such as magnetic susceptibility, thermal properties, photochemistry, and bonding.

Future directions

There are several problems that limit the application of tunnelling spectroscopy, but these can and will be solved in the future. The most critical need is a wider selection of barrier materials to be used as substrates in IETS. The easily grown alumina and magnesia insulators most commonly used in tunnelling studies are virulent redox agents, but methods for producing ultra-thin and chemically passive insulators are now being developed by several groups of researchers.

A workable theory of IETS intensities is also needed. At the moment it is not even possible to correctly predict the relative intensities of two species differing only by isotopic substitution[7]. Finally, it would be desirable to eliminate the top metal deposition step. In the near future it may be possible to marry the methods of tunnelling spectroscopy to IETS and provide tunnelling spectra as a function of position on the sample surface. ☐

K.W. Hipps is at the Department of Chemistry, Washington State University, Pullman, Washington 99164-4630, USA. For additional information fill in Reader Service No. 100.

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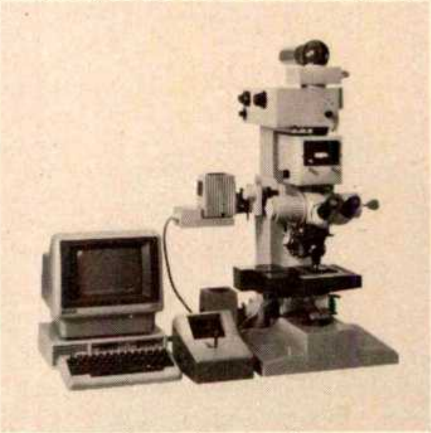
Spectroscopy specifics

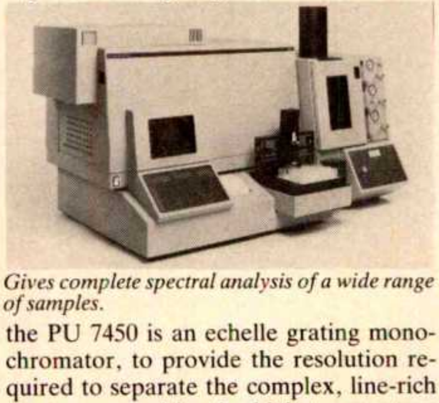
As one of the largest instrumentation shows, the Pittsburgh Conference, held this year 9 – 13 March in Atlantic City, New Jersey, has something for every spectroscopist.

From Carl Zeiss, Inc. comes a **compact microscope spectrophotometer** system designed for forensic work in fibre discrimination, lacquer and paint chip analysis, document identification, and counterfeit analysis (Reader Service No. 101). The $35,000–70,000 (US) Universal Microscope Spectro-Photometer is a modular system for densitometry and spatial analysis of microscopic specimens, and has a spatial resolution in the spectral range from 240 nm (UV) to 2,100 nm (NIR). The system consists of a microscope, photometer, infrared detector, scanning stage and monochromators.



Images as well as analyses samples.

Phillips Analytical has completed its line of spectrometers with its newest offering, the PU 7450 **benchtop inductively coupled plasma spectrometer** (Reader Service No. 102). Phillips says its spectrometer can analyse a wide range of materials from metals and refractories, to geological or biological products. The core of the PU 7450 is an echelle grating monochromator, to provide the resolution required to separate the complex, line-rich spectra created by the high temperature ICP source. All wavelengths are measured at or very close to the blaze angle to achieve high dispersion without loss of efficiency. The plasma source is a 27.12 MHz, 2.5 kW tuned oscillator, chosen to accommodate a wide tolerance of sample types. For routine analysis, the spectrometer can determine up to 20 elements in each stored program, with a total capacity of three stored programs.



Gives complete spectral analysis of a wide range of samples.

The modular UVIKON EASY-10 **spectrophotometer** by Kontron Instruments combines a double-beam spectrophotometer, a personal computer, EASY-10 software and a four-colour plotter (Reader Service No. 103). The system's data handling capabilities include scanning and time drive for rate assays, and kinetic calculations using linear regression.

A convenient spectrophotometer for small laboratories.

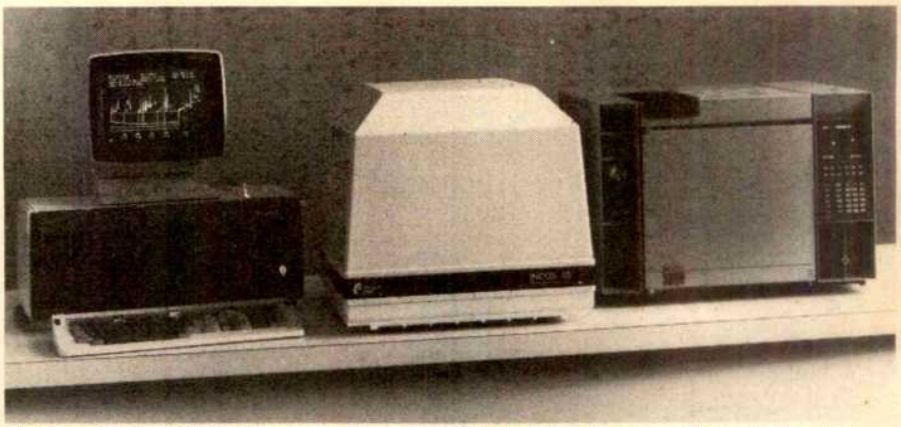
Varian has extended its SpectrAA range of atomic absorption spectrometers to include two new instruments equipped with **Zeeman background correction,** for samples exhibiting structured background signals and spectral interferences (Reader Service No. 104). The SpectrAA 30 and 40 Zeeman spectrophotometers' accuracy of correction is enhanced by using polynomial interpolation of the data points and an extremely rapid duty cycle, to ensure rapid changes in both slope and values of background peaks are accurately monitored. The SpectrAA 40 Zeeman offers an advantage over the SpectrAA

Varian now offers Zeeman background correction in its spectrophotometers.

Zeeman 30 because it is designed for unattended multi-element analysis, and automatically selects the appropriate hollow cathode lamp and warms up the next lamp in readiness for the following element in the sequence. Both systems are floppy disk-based, and have unlimited data storage. Up to 12 stored methods can be selected for multi-element analysis, and all instrumental conditions are automatically set as each method is recalled.

Fluorescence lifetime and transient spectroscopy is now possible with a commercial instrument (Reader Service No. 105). Edinburgh Instruments' PC-based system combines the functions of a multichannel analyser with a computer for reconvolution analysis of decay data. The system interfaces with all existing time-correlated single photon counting instruments through a BNC connection to the TAC. Multichannel scaling is included with options to extend up to 8K data channels and external polarizer control.

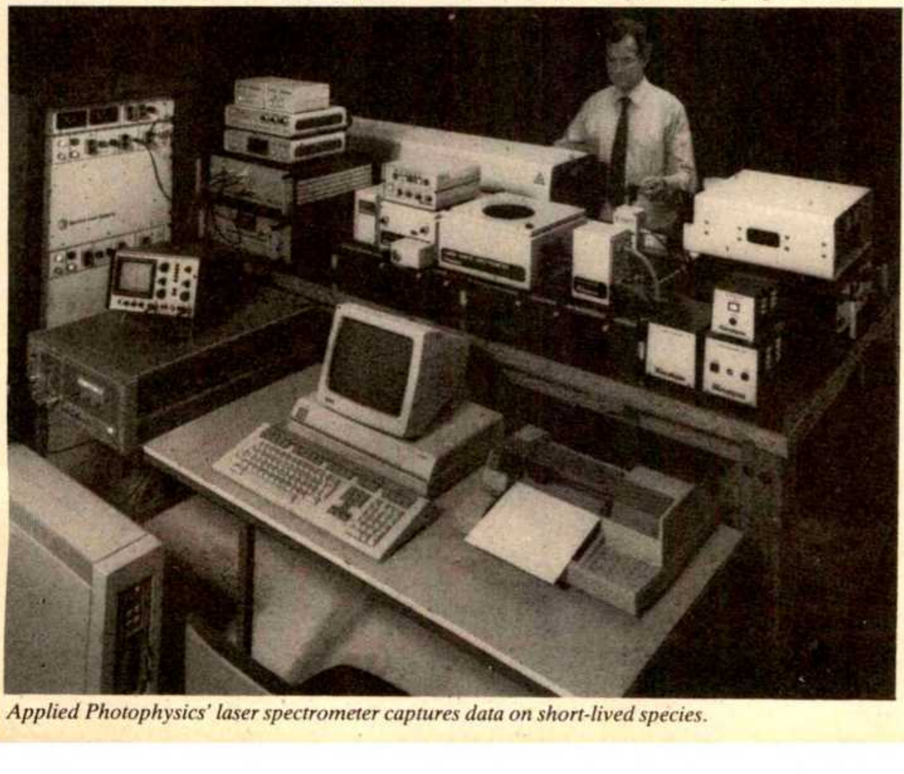
Short-lived species such as radicals, ions and excited states are not quick enough to elude detection by Applied Photophysics' **laser spectrometer** (Reader Service No. 106). Its latest machine overcomes the problem of producing such species in sufficient concentrations for detection by using a high-powered pulse laser to generate new chemical species in a few nanoseconds. Applied Photophysics claims that chemical reactions that last only one hundredth of a millionth of a second can be studied with the new machine. The computerized data collection facilities of the laser spectrometer allow spectra in the UV and visible region to be captured and complex decay kinetics to be analysed.



Finnegan MAT's INCOS 50 puts gas chromatography/mass spectroscopy right on the benchtop.

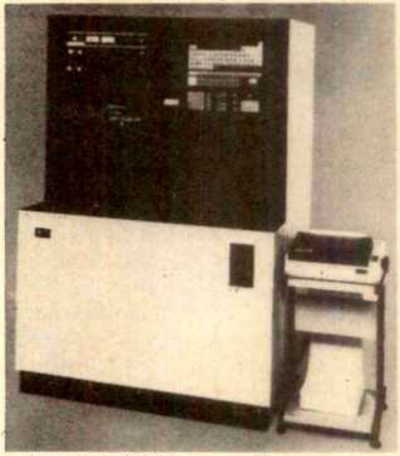
One of Finnigan MAT's newest spectrometers, the INCOS 50, will be making its first US show appearance (Reader Service No. 107). The INCOS 50 is a **benchtop-sized gas chromatography/mass spectrometer** system that Finnigan MAT claims is ideal for laboratories performing environmental monitoring and pre-employment drug screening. Its $77,000–85,000 (US) price has made it Finnigan MAT's fastest growing product family.

Beckman says that its DU Series 60 **UV-visible spectrophotometers** fit virtually any application in bioresearch and quality control (Reader Service No. 108). Its $6,700–8,200 (US) scanning spectrometers can provide final values for protein assays, DNA purity and tablet dissolution studies, kinetic and quantitative assays, wavelengths, gel and film scanning, microsampling analysis, and method determination. The $14,500 (US) DU Series 70 goes one step further by incorporating a CRT display, and by accommodating a range of sampling accessories.



Applied Photophysics' laser spectrometer captures data on short-lived species.

For routine analytical use, Rigaku offers its model 3030 **X-ray spectrometer** (Reader Service No. 109). Rigaku says its $84,825 (US) system is capable of analysing all elements from fluorine through uranium, and only requires the operator to press the corresponding element button on the periodic chart panel to initiate the analysis. All analytical parameters and correction factors for groups of up to 15 elements may be preprogrammed, and corrections are automatically made for background, matrix absorption, enhancement, and line overlap. For automatic transmission of analytical data to an external computer, the RS232C interface is available.

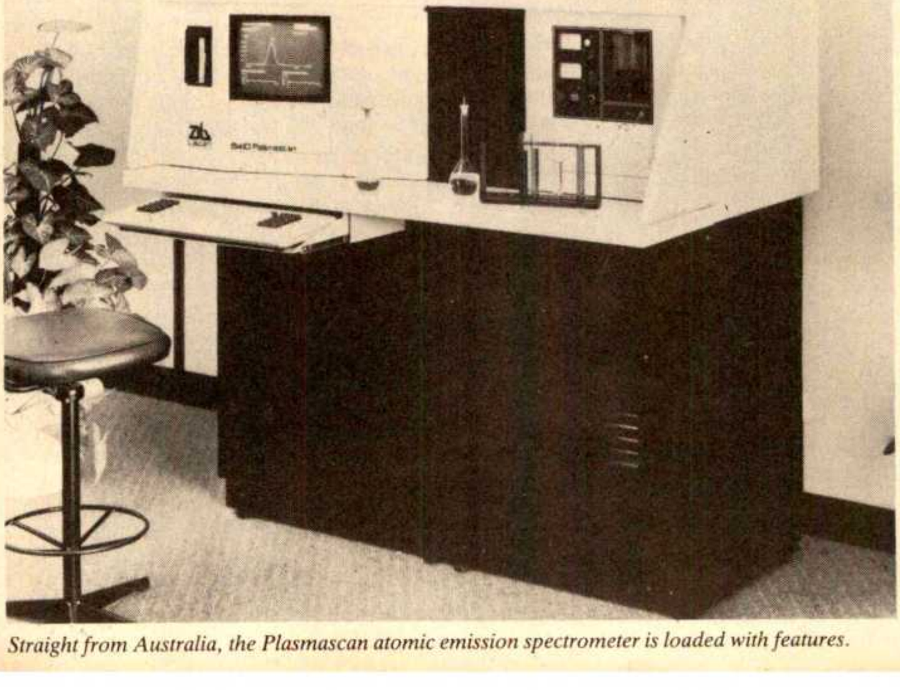


Rigaku's Model 3030 uses an X-ray.

Baird & Tatlock Ltd is expanding its range of **UV-visible spectrophotometers** by now distributing Gilford Response spectrophotometers (Reader Service No. 110). The microprocessor-controlled scanning instruments cover an operating range of 200–750 nm, and offer both single and temporal dual-beam readings. The results are displayed on a CRT in both graphic and tabular format, and the operator can choose parameters and manipulate collected data by the same means. The spectrophotometer includes programs of wavelength scanning, kinetics, time scanning, multiwavelength analysis, standard curve plotting and read samples. The photometer calculations available range from absorbance and first and second derivations to concentration, absorbance ratio, enzyme rate, peak identification, area integration and multiwavelength analysis at five wavelengths. Other models in the series feature automatic positioning of a six-place cuvette holder, twin disk drives for archival data and program storage and additional software for multicomponent analysis and thermal denaturation-renaturation analysis.

The JMS-HX 100, by Jeol, is a **high-performance mass spectrometer** which can be used in MS-MS tandem operations (Reader Service No. 111). The system is also suited for fast atom bombardment ionization techniques, for the measurement of compounds with mass numbers between 3,000 and 6,000, including biological compounds such as glycolipids, proteins and peptides. In its MS-MS mode, the JMS-HX 110 can determine an amino-acid sequence of a polypeptide and perform quantitative analysis of trace samples. Masses of 12,500 daltons at a maximum acceleration voltage of 10kV, up to masses of 125,000 daltons at 1kV can be covered. A broad range of ion sources, including EI/CI, EI/CI/DCI, FAB/EI/CI/DCI and FAB/FD combinations are available. According to Jeol, its system has a sensitivity of 0.1 ng of methyl stearate in the EI mode and an ion intensity of 3×10^{-8} coulomb μg^{-1} with the molecular ion of methyl stearate. The JMS-HX 100's data system corrects all parameters for runs in either positive or negative, controls temperature, and acquires, tabulates, and displays spectra in several ways including three-dimension chromatograms.

Techmation is distributing the Plasmascan 8410 **atomic emission spectrometer** manufactured in Australia by Labtam (Reader Service No. 112). Special features of the Plasmascan spectrometer include a demountable torch for ease in cleaning, and a stop/flow GMK nebulizer designed to tolerate many difficult matrices with a high solid content. The alignment of the nebulizer is not critical and its design eliminates particle build-up at the gas/liquid interface. The Plasmascan's integral computer runs Spectrometer Laboratory Information Management Package (Slimpac) software. A vacuum monochromator with a range of 160 to 820 nm is standard, and systems can be supplied with one or two monochromators fitted as required.

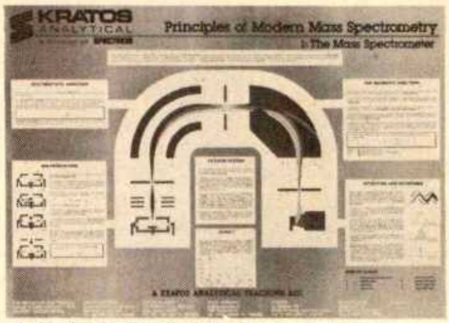


Straight from Australia, the Plasmascan atomic emission spectrometer is loaded with features.

Literature and more

An application note entitled *Thin Film Analysis XPS Depth Profiling* is available from Kratos Analytical (Reader Service No. 113). The free publication explains **depth profiling by X-ray photoelectron spectroscopy**, a technique finding wide application in quality control laboratories where assessments of surface contamination and thin film analyses are performed.

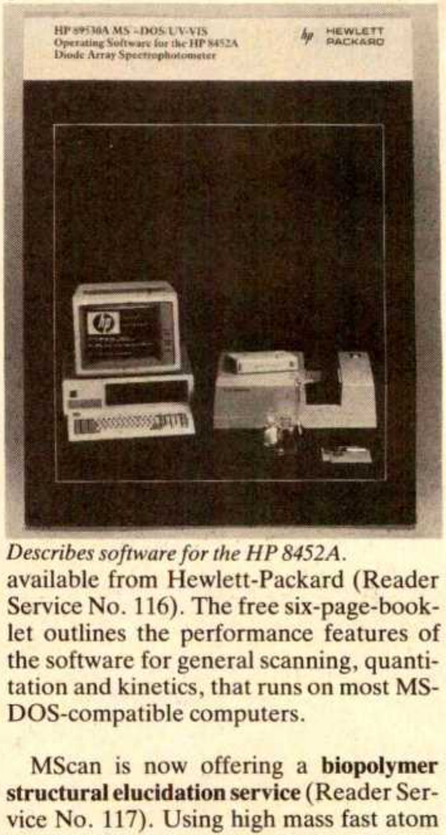
A 24-page brochure describing SPEX Industries, Inc.'s full line of **light measuring instruments** and accessories is now available (Reader Service No. 114). The brochure includes spectrometers, spectrographs and control systems, and also provides general information to help users decide which type of instrument system best meets their specific analytical needs.



A good reference for the lab or the classroom.

A **full-colour wallchart** available from Kratos Analytical details the essential elements of mass spectrometer design and function (Reader Service No. 115). The chart describes a mass spectrometer with full reference to its constituent parts; ion source, analyser, detector and vacuum system. The free wallchart is intended as both a teaching aid and a memory prompter for laboratory personnel, and also outlines the electrostatic analyser, the magnetic analyser, ion production and detection, and recording.

Software made especially for the HP 8452A diode-array UV-visible spectrophotometer is described in a new brochure available from Hewlett-Packard (Reader Service No. 116). The free six-page-booklet outlines the performance features of the software for general scanning, quantitation and kinetics, that runs on most MS-DOS-compatible computers.


HP 89530A MS-DOS UV-VIS
Operating Software for the HP 8452A
Diode Array Spectrophotometer
hp HEWLETT PACKARD

Describes software for the HP 8452A.

MScan is now offering a **biopolymer structural elucidation service** (Reader Service No. 117). Using high mass fast atom bombardment mass spectrometry, MScan will perform protein and carbohydrate analysis, solving a variety of structural problems including identification of N- and C-terminal blocking groups, checking the integrity of synthetic peptides, and identification of post-translational modifications such as position and type of carbohydrate–protein linkages.

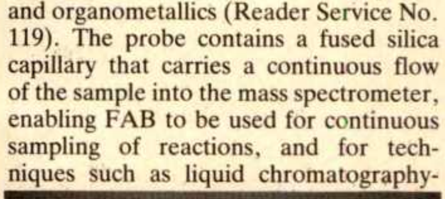
Heyden & Son Ltd has announced the publication of the *Sadtler Infrared Spectra Atlas of Rubber Chemicals* (Reader Service No. 118). A compilation of infrared spectra, physical and chemical data, and supporting information for 589 rubber

Bio-Rad offers a variety of optical benches that reaches from near- to far-IR.

chemicals grouped by principal function, the **spectra atlas** provides a practical desk reference in a single volume. Spectra are arranged one per page, and FT-IR spectra are presented in a transmittance versus frequency (wave number) format generally over the spectral region 4,000 to 450 cm^{-1}. Each compound is listed by its commercial or trade name, and accompanying information includes the classification, manufacturer, properties, function or use, and the method used in sample preparation.

Spectroscopy aids

Kratos Analytical has announced a **continuous flow fast atom bombardment (FAB) probe** to broaden the application of mass spectrometry to allow the analysis of non-volatile compounds such as peptides and organometallics (Reader Service No. 119). The probe contains a fused silica capillary that carries a continuous flow of the sample into the mass spectrometer, enabling FAB to be used for continuous sampling of reactions, and for techniques such as liquid chromatography-mass spectrometry. The probe also increases the sensitivity of FAB mass spectrometry by reducing background peaks. The new probes are now available for the Kratos MS50 and MS890 FAB mass spectrometry units, and existing Kratos instruments can be retro-fit for the new probe, if desired.



The Kratos Analytical FAB probe is useful for non-volatiles.

Bio-Rad is offering **optical benches** for near-IR, far-IR and vacuum work for the Digilab FTS-40 and FTS-60 spectrometers (Reader Service No. 120). The FTS-40N/FTS-60N near-IR benches feature tungsten halogen sources, quartz beam splitters and PbSe detectors, and have ranges from 400 to 15,000 cm^{-1}. The far-IR benches are equipped with Hg sources, Mylar beam splitters and DTGS detectors, giving them a range down to 10 cm^{-1}, and making them suitable for organometallic, catalyst and zeolite analysis. For superfine work in the far-IR, variously equipped vacuum benches are available. Bio-Rad claims a baseline peak-to-peak signal-to-noise ratio of 1,400:1 for a one second collection time at 1 cm^{-1} resolution for its benches.

Perkin-Elmer has developed its AS-80 Autosampler as a stand-alone computer-controlled **multipurpose sampling system** for the Model 5100 flame atomic absorption spectrophotometer, and the Model 6500XR and Plasma II ICP Emission Spectrometers (Reader Service No. 121). The system has random access sampling capabilities, and can also be used in a robotics system for a wide variety of applications. Features of note include interchangeable sample trays, automatic electronic identification of the sample, high sampling frequency, and solvent and acid resistant sampling components.

For reproducible analyses of aqueous-based or organic samples that are not accomplished easily with transmission infrared spectroscopy, Spectra-Tech, Inc. introduces its QC Contact Sampler as a **horizontal ATR accessory** (Reader Service No. 122). With the $3,500 (US) QC Contact Sampler, the crystal under study is mounted and sealed into removable steel plates in a horizontal position with the main sampling surface facing upward. This design permits a rapid interchange of crystals to provide different angles of incidence for depth profiling or varying effective pathlengths without the need for optical realignment. Spectra-Tech's sampler is geared toward quality control applications, and can be used for the analysis of liquids, powders, pastes, semisolids and films. ☐

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Occupational trends in the United States

Richard Pearson

US government figures show just how rapidly employment patterns are changing.

In September last year the Employment column reported on the occupational changes expected in the United Kingdom over the period to 1990[1]. That study was unique in being based on the expectations of employers and as such it was able to explain many of the underlying causes of change. More frequently, however, economic models or statistical projections are used to map out future occupational change. Their utility is largely dependent on the availability of historical time series of occupational data, complemented by a variety of desk research techniques. The most developed statistical projections of occupational change are those produced biennially by the Department of Labor in the United States. One of the main outlets and uses of the model is in the production of the annual *Occupational Outlook* publication which details occupational and training profiles of over 750 occupations. The underlying model itself draws an extensive set of time series data on 150 industries and 750 occupations making it the most sophisticated analytical econometric occupational model in the world.

Table 1 Occupations with the largest job growth, 1984-95 (numbers in thousands)

Occupation	Change in employment 1984–95	
	Number	Per cent
Cashiers	556	29.8
Registered nurses	452	32.8
Janitors and cleaners	443	15.1
Truckdrivers	428	17.2
Waiters and waitresses	424	26.1
Wholesale trade	369	29.6
Nursing aides, orderlies, and attendants	348	28.9
Salespersons, retail	343	12.6
Accountants and auditors	307	34.8

Data from ref. 2.

From a base of 107 million in 1984, total employment in the United States is expected to grow to 130 million in 1995, a growth of about 15 per cent. This represents a slowdown in the growth of the previous decade. Service industries which account for 7 out of 10 jobs are expected to continue to grow more rapidly than manufacturing and other industries. This growth will be fuelled by rising living standards, in turn leading to greater demand for health care, entertainment, banking and insurance services, and these sectors are expected to account for nine out of ten new jobs over this period. Employment in US goods producing sectors is also expected to grow, albeit at a much slower rate of only about 6 per cent. This growth rate, however, in part represents a recovery after the recession of 1981–82 and in 1995 employment is likely to be only slightly higher than that in 1979. The main engine of this growth is expected to be increasing capital spending and defence expenditure, with the computer, materials handling equipment and scientific and instrument industries particularly benefiting and showing output growth significantly in excess of projected productivity gains. The sectors showing decline include basic industries such as mining, where productivity gains, improvements in technology and in some activities rising import penetration will reduce employment. Agricultural employment is also expected to continue its long-term decline (Fig. 1).

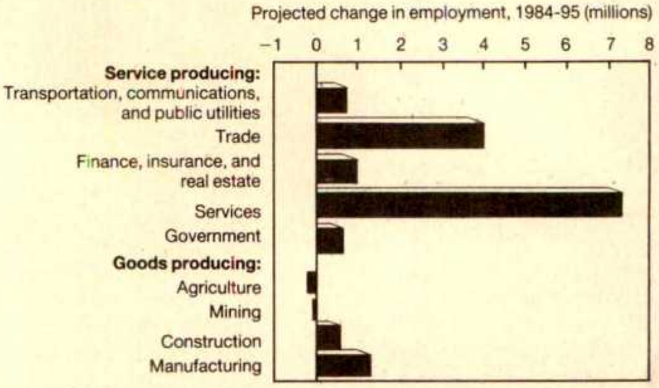

Fig. 1 Data from the US Department of Labor study show that some industries will grow much faster than others during the 1990s.

One thing that is not clear from these figures, though, is the extent to which they represent a statistical name change. In the United Kingdom, for example, almost half the growth in service jobs (actual and projected) through the 1980s is expected to be due to the goods producing sectors 'contracting out' activities such as wholesaling, catering, transport and professional services to specialist service sector companies. Thus a crude interpretation of the figures, in the UK case at least, overemphasizes the so called decline of manufacturing and the growth of services. But it is not clear whether the United States is following a similar trend.

A more balanced perspective can be had from an assessment of occupational change, although these figures are in part derived from industrial or sectoral trends. The occupations with the highest growth rates will be paralegal, computing and electronics staff, and medical assistants all showing increases of 50 per cent or more in employment levels. In absolute terms, then cashiers, nurses, cleaners, truck drivers and waiters are all groups expecting to expand their employment by 400,000 jobs or more (Table 1). A detailed analysis shows no general upgrading of jobs to graduate status, there being no more growth among jobs requiring extensive training than those requiring little formal training, and only one in four of the new jobs is expected to require a college degree. The occupations showing the largest decline are stenographers (down 40 per cent), shoe machinists (down 32 per cent), and railroad operators (down 26 per cent), these are to be found either in the declining sectors or are being severely affected by technological change, the stenographers being a prime example.

For scientists and engineers the best prospects are for those related to computing and electronics, and engineers in general are expected to have better job prospects than scientists although both groups are expected to show at least average employment growth. Military technology, the automation of industrial production, the development of energy sources, basic research, and environmental protection are all activities generating improved employment prospects for such staff.

Overall the rate of population growth in the United States is slowing down and the total labour force is expected to grow a little slower than will employment opportunities. The final balance in 1995 will be subject to the many uncertainties in the economy, as well as the rate of technological change, and changing social habits and priorities. Nevertheless, the outlook in terms of job opportunities is expected to continue to improve, and will encompass a wide range of skill levels and locations. Flexibility will be the key need for job seekers operating in such a rapidly changing market. □

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1. Pearson, R. *Nature* **323,** 94 (1986).
2. *Occupational Projections and Training Data* (US Department of Labor, 1986).

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CORE STAFF POSITIONS

The International Council for Research in Agroforestry (ICRAF) with headquarters in Nairobi, Kenya, is recruiting the following:

1. ***FIELD CROP AGRONOMIST***
   To help develop field research with tree/crop mixtures, and for on-farm agroforestry investigations. At least 10 years experience and a PhD required. Competence in field experimentation/statistics essential.
2. ***APPLIED STATISTICIAN/BIOMETRICIAN***
   To help develop appropriate experimental designs/analysis procedures for investigations with multipurpose trees and tree/crop mixtures. Also to advise and backstop ICRAF's research programmes. At least 10 years experience and preferably a PhD required.
3. ***TRAINING OFFICER***
   Within ICRAF's collaborative programmes with national institutes to plan, manage and conduct training courses, seminars and workshops (particularly in Francophone Africa), to prepare curricula for training/education programmes and to develop training materials. Postgraduate degree in agriculture, forestry, or similar plus 10 years experience with excellent English/French.

All three positions are based in Nairobi and are on international terms with a generous benefit package and salary commensurate with experience/qualifications.

Further details available on request from the Director, ICRAF, P.O. Box 30677, Nairobi. Closing date 30th April 1987.
(W3871)A

THE UNIVERSITY OF KUOPIO

invites applications for a position as

PROFESSOR OF BIOTECHNOLOGY

The University of Kuopio is wide-ranging in its subject areas relating to Health Care and Environment Studies. In addition to Pharmacy, Dentistry and Medicine, the Natural and Social Sciences are available for study. The instruction began in 1972 and at present there are about 2,000 students aiming at the basic university degree and about 400 postgraduate students. The University has a staff of nearly 700 faculty and support personnel. Biotechnology will be offered as a new undergraduate 4-year program (M.Sc. level).

Applicants for the advertised position should show excellence in research and have a capacity for teaching undergraduate and graduate students and to further develop this area in the University, and would be expected to possess a degree in Biochemistry, Molecular Biology, Microbiology, Medicine, or a related scientific discipline. An interest in interacting with industry would be highly regarded, as would appropriate experience in one or more of the major areas of Biotechnology.

Salary (S 27) is at present in the range of FIM 11,531 to 15,152 per month but is negotiable. The appointment is made for a maximum of 5 years at a time or is open-ended, and is available from September 1, 1987.

The closing date for applications is March 31, 1987. Further information can be obtained from Professor Juhani Kärjä, Rector of the University (phone 358-71-162100), or Professor Pekka Mäenpää (phone 358-71-162494). The applications including curriculum vitae, list of publications, statement of research interests and names of three persons who can be contacted for references should be sent to **University of Kuopio, Administration, P.O. Box**

UNIVERSITY OF TORONTO

Department of Clinical Biochemistry and Banting and Best Diabetes Centre

A FACULTY POSITION is available for a molecular biologist interested in growth factors. The applicant must have a strong background in the molecular and cell biology of growth factors or their actions and is expected to develop an independent research program in growth factors in an environment that includes investigators concerned with the vascular complications and genetics of disease. The laboratory will be in the newly built research wing of the Toronto General Hospital which is at a central location proximal to the University and other hospital research institutes. The position will include a University appointment in the Department of Clinical Biochemistry at a rank and salary commensurate with experience.

Please send your curriculum vitae, a brief letter describing your accomplishments and goals and the names of at least three referees to: **Chairman — Search Committee, Banting and Best Diabetes Centre, 92 College Street, Toronto, Ontario, Canada M5G 1L4.**

In accordance with Canadian immigration requirements, first consideration will be given to Canadian citizens and permanent residents of Canada. Both men and women are encouraged to apply. (NW588)A

POSTDOCTORAL POSITION

now available in biochemistry. We are an established group in the area of membrane structure and function, magnetic resonance spectroscopy and biophysics, with interests in signal transduction, protein kinases, phospholipase c, and molecular basis of tumor promotion. We are looking for a colleague with mutual interests and a strong background in biochemistry. Cloning experience helpful. Send vitae and names and addresses of three references to: **Dr O. Hayes Griffith, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403** by April 15, 1987. Telephone: (503) 686-4634. *An Equal Opportunity/Affirmative Action Employer.*
(NW564)A

DEVELOPMENTAL OR CELL BIOLOGIST

The Department of Anatomy and Cell Biology invites applications for a tenure-track position at the assistant or associate professor level. Candidates for professor may be considered. Individuals at the assistant professor level should have postdoctoral experience and will be expected to develop a strong independent research program with external funding from national sources. At the associate professor level, candidates must be principal investigator of a nationally funded program. While the direction and focus of the prospective candidate's research is open, we are seeking individuals who are asking important questions in developmental and/or cell biology and who are using modern research tools, preferably molecular genetics, to address these questions.

The successful candidate must be committed to participating in an active graduate program, and will be expected to teach medical students in a team-taught histology course. The successful candidate need not have prior histology experience, but must be willing to learn and help teach the subject during his/her first year. Please send a curriculum vitae with the names, addresses and telephone numbers of 3 referees to: **Dr Peter Stambrook, Department of Anatomy and Cell Biology, University of Cincinnati College of Medicine, 231 Bethesda Avenue, ML #521,**

Physical Organic Chemist

c.£21,000 + bonus

The Career Move...?

This major multinational pharmaceutical company wishes to strengthen further their well established UK development group. Consequently our client now intends to appoint a physical organic chemist to make a significant impact upon the analytical development of prospective drug candidates.

Reporting at Director level and responsible for a team of five people, you will apply all the latest analytical techniques. Prospects for early promotion within this US based multinational are excellent, and just as important, scientific staff are able to retain detailed professional involvement rather than becoming administrators, even at the most senior levels.

You must have a PhD and at least five years experience in the pharmaceutical/fine chemical industries or other organisations employing high precision analytical techniques. As you would expect technical excellence is vital, combined with the personality and ability to perform and achieve at a high level. The strategic importance of this appointment means that for the right person salary is unlikely to be a limiting factor and, naturally, generous assistance is available to relocate to the Home Counties.

Initially, please contact **Larry Botheras** on **(0992) 552552**, or write enclosing a CV and current salary details at **Macmillan Davies, Salisbury House, Bluecoats, Hertford, SG14 1PU.**

(2245)A

Macmillan Davies
INTERNATIONAL SEARCH EXECUTIVE

The Pennsylvania State University

DEAN

College of Science

Applications and nominations are invited for the position of Dean of the College of Science. Candidates should have: credentials to merit appointment as a tenured full professor in the College; experience in teaching, research and administration in an academic environment; demonstrated development and administrative, leadership and management skills.

The position requires an individual who can effectively lead and manage a large and complex college. The College of Science (one of ten colleges in the University) provides instruction and research opportunities in the biological, mathematical and physical sciences to 2800 baccalaureate and 650 graduate degree students. There are 412 faculty in the college at the University Park Campus, and 287 at the University's 21 campuses located throughout the Commonwealth. The budget for 1986-87, including sponsored research, is $36 million. In addition, annual private gifts total about $5 million.

The Search Committee will begin to review applications in April and continue to receive them until a candidate is selected. Nominations and letters of application accompanied by a curriculum vitae and other supporting materials, should be mailed to:

Dr. Stephen J. Benkovic, Chair
Science Dean Search
The Pennsylvania State University
Room 201 Old Main, Box NM
University Park, Pennsylvania 16802

(NW599)A

WE ARE AN AFFIRMATIVE ACTION, EQUAL OPPORTUNITY EMPLOYER AND ENCOURAGE WOMEN AND MINORITIES TO APPLY.

PROFESSOR AND CHAIRPERSON

Department of Microbiology and Molecular Genetics

UMDNJ-New Jersey Medical School is seeking applications for the position of Professor and Chairperson of the Department of Microbiology and Molecular Genetics. Candidates must have outstanding academic credentials with demonstrated abilities in research, teaching and administration. Send curriculum vitae with the names of three references to:

Gerson Weiss, M.D.,
Chairman, Microbiology Search Committee,
UNIVERSITY OF MEDICINE AND
DENTISTRY OF NEW JERSEY-NJMS
Department of Obstetics and Gynecology,
185 South Orange Avenue,
MSB-E506, Newark, N.J. 07103-2757.

An equal opportunity/affirmative action employer m/f/h/v. (NW586)A

UMD

C·A·B INTERNATIONAL

CAB International Institute of Parasitology
requires a

NEMATOLOGIST

The Institute, situated 20 miles north of London, on the Field Station of the London School of Hygiene and Tropical Medicine at St. Albans, is one of a group of scientific centres of CAB International, an international organisation supported by 29 Commonwealth Governments.

The Institute provides a world service on the identification of plant and insect nematodes as well as animal and human helminths. Taxonomic and other research projects and surveys on these parasites, particularly those of economic importance, are also undertaken and the Institute also provides training facilities and information relating to parasitology from the world's literature, mostly through its publications.

DUTIES: The main duties will be the identification of plant-parasitic nematodes. The successful applicant will be expected to specialise in particular groups of these nematodes, to undertake taxonomic research and become expert in them. The applicant will also, if required, assist in the information and training activities of the Institute.

QUALIFICATIONS: A PhD or research degree in which taxonomy, parasitology or agricultural zoology has featured is preferred. The applicant should have a genuine interest in nematode taxonomy and the ability to carry out research and publish the results. Graduates who can demonstrate an interest in the above are also encouraged to apply.

Experience or an interest in biochemical, ultrastructural, or numerical approaches to taxonomy will be an advantage.

SALARY: The appointment will be on the scale of £6,711—£9,225 or £8,405—£11,323, according to age and qualifications, with the prospect of promotion to higher scales (maximum £19,465); plus Outer London Weighting of £615 per annum.

Application form and further details can be obtained from The Director, CAB International Institute of Parasitology, 395A Hatfield Road, St. Albans, Herts AL4 0XU.

Closing date for applications 3rd April 1987. (2250)A

— IRELAND —

DEVELOPMENT SCIENTIST

LABORATORY METHODS

Avondale (Brinny) Chemical Company, a subsidiary of Schering-Plough Corporation, New Jersey, U.S.A. has recently completed a major capital intensive construction project near Bandon, Co. Cork, Ireland to provide the most up-to-date Laboratories and equipment for the manufacture and testing of Intron A, Schering Ploughs brand of alpha-2 Interferon for worldwide distribution. Part of the laboratory program involves method development to maintain our position at the leading edge of technology and to facilitate the scale up of activities to supply a growing market.

The work will involve: Optimization of existing methods, development and integration of new methods into the QA system, trouble shooting within QA and where required with other departments.

The position will require a dynamic person with wide ranging interests and strong academic qualifications. He/she should be capable of working independently and able to co-operate and communicate with multidisciplinary teams. Expertise in tissue culture, skill and appreciation of analytical techniques such as HPLC, Electrophoresis and Radioimmunoassays is required. Familiarity with the problems associated in processing large numbers of laboratory samples would be a strong advantage.

Applicants should write to M. Greene giving full details of career experience at **Avondale (Brinny) Chemical Company, Brinny, Innishannon, Co. Cork.** (2285)A

AUSTRALIA

CSIRO

RESEARCH FELLOW

A$28,107 – A$41,339

DIVISION OF MINERAL PHYSICS AND MINERALOGY

CANBERRA ACT

GENERAL: The Division of Mineral Physics and Mineralogy conducts research in minerals and petroleum exploration as part of the CSIRO Institute of Energy and Earth Resources. A wide spectrum of scientific disciplines contributes to geophysical and geochemical research conducted in collaboration with the mining and exploration industry.

Under a joint arrangement between CSIRO and Bureau of Mineral Resources, research is carried out to establish a practical and economical method, based on microbiological processes, for the enhancement of recovery of residual oil from Australian reservoirs. The project team currently draws on expertise from the Baas Becking Geobiological laboratory. The project has been under way for six years and field tests will soon commence. The successful applicant will be employed by CSIRO and will be located in Canberra.

DUTIES: The appointee will lead a small group and be responsible for designing and conducting experiments to select surfactant-producing bacteria that can be used in field tests and will evaluate such tests.

QUALIFICATIONS: A PhD or equivalent qualification in microbiology with an emphasis on microbial ecology or physiology. The appointee's skills and experience will be in the field of microbial ecology or physiology. Experience with surfactant production by bacteria, or with the microbiology of deep groundwaters, would be an advantage, as would experience in industrial applications of microbiology. Experience in technology transfer to industry would be an advantage.

TENURE: A term of two years with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No M8742, should be directed to:

The Chief
CSIRO Division of Mineral Physics and Mineralogy
PO Box 136
NORTH RYDE NSW 2113
AUSTRALIA

by April 3, 1987

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W3872)A

MOLECULAR AND CELLULAR ASPECTS OF REGULATORY BIOLOGY

The Banting and Best Department of Medical Research, University of Toronto, is seeking candidates for the position of Assistant Professor (Tenure stream). The position is available July 1, 1987 and will carry an initial appointment of five years at a competitive salary. Candidates should have a strong research background and be prepared to develop a research program in Molecular and Cellular aspects of Regulatory Biology (for example - Molecular Endocrinology, Mechanisms of Signal Transduction, Regulation of Gene Expression). The successful candidate will be expected to attract independent research grants, to spend at least 75 per cent of his/her time in research and to participate in graduate and undergraduate training.

Applicants should forward a curriculum vitae, an outline o research interests and three letters of reference to: Dr David H MacLennan, Professor and Chairman, Banting and Best Department of Medical Research, University of Toronto, 112 College Street, Toronto, Ontario, Canada M5G 1L6. In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada, bu foreign applications are encouraged. (NW577)A

Head of Pharmacology

Excellent package + Car

Our client is one of the largest and most prestigious British pharmaceutical companies with an outstanding reputation and substantial investment in research. It is now seeking an outstanding young pharmacologist to manage a team of 35 highly qualified scientists working in a number of therapeutic areas.

Reporting to the Head of Biology, you will ensure that a high standard of original and inventive pharmacology is achieved which effectively contributes to the drug discovery programme. You will be an initiator of scientific ideas and have sound scientific judgement. Above all, you will be a natural leader with good communication skills and the ability to motivate a large team. An aptitude for encouraging and implementing change is also crucial.

Candidates will be men or women aged 32-38, with a good first degree in Pharmacology/Physiology and a Ph.D in Pharmacology. At least six years working experience in the pharmaceutical industry is essential. Of this, a minimum of two years will have been in a man-management role. Multidisciplinary project experience is important and candidates with experience or interest in receptor pharmacology and biochemistry will be very well regarded. In addition, candidates should have experience of taking a compound through a full pharmacological development programme.

An excellent package, including a car and relocation assistance will be negotiated.

For further information, please contact:
Dr John M Padbury, Knight Chapman Limited,
11 Garrick Street, Covent Garden,
London WC2E 9AR. Telephone: (01) 379 7879.

(2279)A

Knight Chapman
Limited
SEARCH AND SELECTION CONSULTANTS

Specialist in PROTEIN CHEMISTRY

Director, Macromolecular Structure, Sequencing and Synthesis Facility.

The Department of Biochemistry at Michigan State University is seeking a Specialist o supervise operation of the MSU Macromolecular Structure Facility. This facility is currently equipped with a Beckman 890M iquid phase sequencer, the Waters PicoTag amino acid analysis system, an Applied Biosystem 380B DNA synthesizer, and a peptide synthesizer. Familiarity with his instrumentation, its operation, and inerpretation of resulting data is required. Additional responsibilities include overall managment of the facility, supervision of echnical personnel (currently two) working in the laboratory, and consultation with users of the facility with respect to development of analytical protocols. Applications rom candidates having a Ph.D. degree with extensive experience operating sequencing and/or synthesis instrumentation is desired. However, candidates having a B.S. degree in biochemistry or related areas with subsequent experience in relevant analytical procedures will also be considered. Salary is negotiable and based on qualifications and experience. An excellent ringe benefits program is provided to university employees.

Send application along with curriculum vitae and two supporting letters of recommendation to: Dr. John Wilson, Department of Biochemistry, Michigan State University, East Lansing, Michigan, 48824. Michigan State University is **an affirmative action/equal opportunity employer.**

(NW569)A

Vacancy Announcement

Associate Vice President for Research

UNIVERSITY OF IDAHO

The University of Idaho invites nominations and applications for the position of Associate Vice President for Research. The Associate Vice President for Research reports to the Vice President for Academic Affairs and Research and is primarily responsible for promoting excellence in research and scholarly activity, enhancing the research environment, providing leadership in broadening and strengthening research and scholarly activity, establishing an administrative structure appropriate for coordinating and supporting research, formulating and administering policy governing research, and representing the university in research-related matters. The Associate Vice President for Research will work closely with the academic deans to coordinate research in the colleges, and with the designated Board of Deans for the various research institutes and centers.

The University of Idaho is a publicly supported comprehensive land-grant institution with principal responsibility for performing research and for granting PhD degrees in Idaho. Regional programs in medical and veterinary medical education in which the state participates are also part of the University of Idaho responsibilities. The UI has programs at 50 sites state-wide. Its land exceeds 8,000 acres. Its nine colleges and Graduate School make the University of Idaho the only comprehensive institution of higher learning in the state.

Applicants or nominees must have an earned doctorate, a distinguished teaching and scholarship record appropriate for appointment as full professor, excellent communication skills, demonstrated ability in obtaining competitive outside funding for research, and administrative experience.

The salary is commensurate with qualifications and experience. The position is available July 1987. Search and selection procedures will be closed when a sufficent number of qualified applicants has been identified, but not earlier than April 10, 1987. Each applicant should submit a letter of application, a current résumé, and the names, addresses, and telephone numbers of three current professional references. References will be treated confidentially and contacted only after preliminary screening is completed. All correspondence should be addressed to: **Ms. Patricia Houle, Secretary of the Associate Vice President for Research Search Committee, College of Engineering, University of Idaho, Moscow, Idaho 83843.**

The University of Idaho is an AA/EOE.

(NW516)A

Pathologist

Shell Research Limited
Sittingbourne
Kent

Shell

Shell Research Limited is conducting a large innovative research programme at its laboratories in Sittingbourne, Kent. A major part of this programme is directed at the evaluation of the safety and environmental acceptability of agrochemicals and other types of company products. We have, at present, a vacancy for an experienced Pathologist to join the large group of scientists and support staff currently working in this field.

As a member of a team interacting with research colleagues on site, the duties would include supervision of necropsies and histopathological evaluation of tissues from acute and chronic toxicity studies. Reporting of the findings and acting as Study Director on some projects will be expected.

At least 3-5 years previous experience in an industrial, academic or contract laboratory environment is sought and a veterinary qualification would be a distinct advantage. You should possess, or be eligible for, Membership of the Royal College of Pathologists.

The Research Centre has modern, well-equipped laboratories situated on a 500 acre farmland site. We offer attractive salaries, a subsidised staff restaurant and good sports and social facilities.

Please write or telephone for an application form, quoting reference number 14/87 to:
Shell International Petroleum Co. Ltd.
Recruitment Division PNEL/211
Shell Centre, London SE1 7NA
Telephone: 01-934 2285
Closing date is 19 March, 1987.

(2287)A

PSYCHIATRIC GENETICS PROGRAM
Department of Phsychiatry, Medical College of Virginia/ Virginia Commonwealth University

is recruiting a fulltime

RESEARCH SCIENTIST

interested in applying molecular genetic techniques to neuropsychiatric diseases. This person will join an active research team with a strong emphasis on quantitative genetic methods, with several major psychiatric genetic field studies currently underway. Responsibilities will include supervision of linkage analyses using RFLP's and establishment of immortalized cell lines. The candidate will be strongly encouraged to establish his/her own research program in an area relevant to the molecular biology or neuropsychiatric diorders. Must have Ph.D. or equivalent and advanced research training in molecular genetics. This will be a tenure track position. Send CV and references to **Dr. K. Kendler, Depts. of Psychiatry and Human Genetics, MCV, Box 710, Richmond, VA 23298.** *MCV/VCU is an EEO/AA Employer.* Women and minorities are encouraged to apply.

(NW502)A

NATIONAL INSTITUTES OF HEALTH

Molecular Biology/ Transgenic Mice

Molecular Biologist, M.D., Ph.D., D.D.S., or equivalent, with experience in recombinant DNA technology and construction of vectors to work on transgenic mice and autoimmune diseases. The candidate should have at least three years postdoctoral experience. The initial appointment will be for two years with renewals on a yearly basis. Starting salary range, depending on experience, is $24,000 to $46,000 per year. U.S. citizenship required. The position is available immediately. Send curriculum vitae to:

Dr. Lois Salzman
Assistant Scientific Director
National Institute of Dental Research
National Institutes of Health
9000 Rockville Pike
Building 30, Room 121
Bethesda, Maryland 20892

An Equal Opportunity Employer (NW567)A

POSTDOCTORAL POSITION

Available July 1987 or earlier to study the structure and function of sarcoplasmic reticulum Ca^{2+} release channel or dihydropyridine-sensitive Ca^{2+} channel using biochemical, biophysical, immunological and molecular biological techniques. Applicants should have a recent PhD and have a strong background in either biochemistry, biophysics, cell biology or physiology.

Submit letter of application, curriculum vitae, two reprints or manuscripts of recent experimental work and two letters of recommendation to: **Dr Kevin P. Campbell, University of Iowa, Department of Physiology & Biophysics, 6-530 Bowen Science Building, Iowa City, Iowa 52242 (319) 335-7867.**

The University of Iowa is an Equal Opportunity/Affirmative Action Employer. (NW585)A

POSTDOCTORAL POSITIONS ENZYMOLOGY AND PROTEIN CHEMISTRY

Positions available immediately for individuals with Ph.D.'s in biochemistry, molecular biology or related areas to study two control enzymes of glucose metabolism.

Phosphorylase Kinase (Skeletal Muscle) — Subunit interactions, recognition sites, architecture, reconstitution, structures and roles.

Phosphoenolpyruvate Carboxykinase (Liver) — Active site mapping (GTP and divalent metal binding sites and identification of critical Cys) and catalytic fragments.

Newly remodeled, fully equipped laboratories and competitive salaries. Please send curriculum vitae and names and telephone numbers of three references to: Dr. Gerald M. Carlson, Department of Biochemistry, University of Tennessee, Memphis, The Health Science Center, 800 Madison Avenue, Memphis, TN 38163.

The University of Tennessee is an Equal Opportunity Employer

(NW568)A

YORK UNIVERSITY

FACULTY OF SCIENCE

Department of Biology

Applications are invited for a tenure-stream position, which may be filled at a senior or junior level, depending upon the qualifications and experience of the applicant. We seek a plant cell/molecular biologist with a PhD degree and a background in plant, algal or fungal biology and current research emphasis in cell or molecular biology. An established and vigorous research programme is paramount but teaching ability in plant-related topics is important.

The starting date for this appointment is September 1, 1987. In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. Please send letter of application, curriculum vitae and arrange for three letters of reference to be sent to **Dr A. S. M. Saleuddin, Chairman, Search Committee, Department of Biology, York University, North York, Ontario, Canada M3J 1P3.** Closing date for receipt of applications is May 1, 1987. (NW589)A

Research Scientists
Analytical Development

Celltech, a leader in the field of Biotechnology and a world leader in mammalian cell culture and large-scale monoclonal antibody production, has achieved its present success in six years. Pioneers in several areas, our achievements are based on the foundations of scientific excellence, innovation and commercial expertise.

Two recent postdoctorates or graduates with at least 2 years' relevant experience in one of the biological sciences or chemistry are required to join the Analytical Development group. The successful applicants will be involved in the design, development and validation of analytical methods to meet both R & D and regulatory requirements for the registration of therapeutic proteins.

The first position would ideally suit someone with a background in HPLC of proteins and polynucleotides whilst the second position requires someone with a background in immunology and immunoassay techniques. For both positions a good general knowledge of techniques relating to protein biochemistry, molecular biology and statistical methods would also be beneficial.

Celltech is an ambitious and forward looking company, which places as much emphasis on its people as its products. You'll receive a good salary, with all the benefits you'd expect from working for a large, successful company that's interested in making the most of you.

Please apply, in the first instance to Jane Davenport, P.S.A.S., 100a High Street, Slough, Berks SL1 1HL, quoting ref: 009. (2294)A

CELLTECH

Nestlé

For our new research centre situated north of Lausanne, we are increasing our research efforts in a number of areas and require a suitable candidate with PhD and postdoctoral experience for the post:

Microbial physiologist

to investigate the biochemistry and microbiology of fermented foods with the aim of developing new processes. We seek an individual with a strong background in biochemistry and microbiology as well as proven capabilities for independent research. The successful candidate will be responsible for developing his or her own research programme. Publications are actively encouraged.

Please send your application to: Mrs M. Germanier-Audrin, Personnel Services, Research Centre, NESTEC Ltd., CH-1800 VEVEY, Switzerland. (W3864)A

ELSEVIER PUBLICATIONS CAMBRIDGE

SUBEDITOR

Our widely read monthly review journals *Immunology Today* and *Parasitology Today* need a new Subeditor.

The papers in these journals are commissioned from scientists worldwide. The Subeditor is responsible for turning the manuscripts into print. This requires collaboration with authors to achieve clear, accurate writing and helpful illustrations, and communication with in-house production staff.

We need a person with a good grasp of immunology. Publishing experience is not essential but a familiarity with scientific writing and its assessment is required, combined with an eye for detail and an ability to work accurately under the pressure of monthly deadlines.

The position carries a competitive salary, 21 days holiday and pension fund opportunities. Our organisation of 40 people is located in a pleasant, modern office near the centre of Cambridge.

Handwritten applications, with a c.v., should be sent to:

Dr. J. R. Inglis,
Elsevier Publications Cambridge
68 Hills Road
Cambridge CB2 1LA (2288)A

SENIOR MACROMOLECULAR CRYSTALLOGRAPHER

The LILLY RESEARCH LABORATORIES, a Division of Eli Lilly and Company, has an opening for a Senior Scientist who will collaborate with established groups from several disciplines to study the structure of proteins and their interactions with other biologically active molecules.

Applicants must have a doctoral degree, two or more years postdoctoral experience and the proven ability to determine protein crystal structures. In addition, a strong background in biochemistry and computer programming is essential. Familiarity with other biophysical techniques for studying proteins would be desirable.

The Lilly x-ray crystallography laboratory is exceptionally well equipped for macromolecular studies and includes a state-of-the-art automated protein crystallization system, diffractometers, a rotating anode x-ray generator, an area detector, computers and graphics facilities.

Qualified candidates should send a curriculum vitae to:

Lilly

John W. Crooks
Manager, College Relations (SRS)
Eli Lilly and Company
Lilly Corporate Center
Indianapolis, Indiana 46285

Equal Opportunity Employer

(NW587)A

DALHOUSIE UNIVERSITY

Postdoctoral position to work o molecular mechanisms of mutation by carcinogens. The research involves site-specifi mutagenesis to study the effec of DNA repair on mutation frequencies produced by DNA carcinogen adducts. Applicant should send resumé, two recen papers or manuscripts an names of two referees to: D Robert W. Chambers, Department of Biochemistry, Dalhousi University, Halifax, Nova Scotia Canada B3H 4H7. (NW574)A

POSTDOCTORAL POSITION

Open July 1, 1987.

To work on a National Institut of Health-funded project usin genetic and biochemica analysis to study the mechanism of mitochondrial protein import in yeast. Experience in either yeast genetic or recombinant DNA technology an advantage. Send C and references to: Dr J M Garrett, Department of Biology, Hamilton College, Clinton, New York 13323.

(NW583)A

UNIVERSITY OF THE WEST INDIES

Mona, Jamaica

Applications are invited for the following posts at the Mona Campus:

PROFESSOR OF PHYSICS

Appointment is to be made in the field of one of the Department's major interests. Successful applicant will be expected to organise and teach undergraduate courses and to lead in the further development of research in the area of his speciality. Some background in Electronics would be an advantage. The Department's major interests are: Energy Studies, Materials Science, Atmospheric Physics, Astrophysics, Electronics. Person appointed will be expected to assume duties as soon as possible.

PROFESSOR OF ZOOLOGY

Successful applicant will be expected to shape and teach undergraduate courses as well as to develop the graduate programme and also to lead in the further development of research in the Department.

SALARY SCALES: PROFESSOR In range J$56,479–69,363.

Unfurnished accommodation at rental of J$360 per annum; or housing allowance of 20% of pensionable salary plus 10% Mona housing allowance. UWI contribution of equivalent of 10% of pensionable salary to Superannuation Scheme. Subsidised Health Service. Annual Study and Travel Grant of J$5,230 for self, J$1,743 for spouse, and J$581 each for up to three (3) children. Book Grant up to J$1,500 per annum. Entertainment and Transportation Allowances totalling J$20,400 p.a. (Professor), J$15,500 p.a. (Senior Lecturer), J$14,400 p.a. (Lecturer), J$9,900 p.a. (Assistant Lecturer). Up to five full economy class passages plus baggage allowance up to US$900 on appoinment and normal termination. Special allowance up to US$300 for shipment of academic books and teaching/research equipment on appointment.

Detailed applications (three copies giving full particulars of qualifications and experience, date of birth, marital status, and names and addresses of three referees should be sent as soon as possible to the **Campus Registrar, UWI, Mona, Kingston 7, Jamaica. Further particulars of the posts are available from the same source or from the Secretary General, Association of Commonwealth Universities (Appointments), 36 Gordon Square, London WC1H 0PF,** to whom candidates in the UK should also send one copy of their applications.

(W3875)A

Dalgety U.K. Limited

Biochemist/ Microbiologist

Cambridge

Dalgety U.K. Limited, one of Britain's leading companies in the food and agricultural industry, has an attractive opportunity for a Life Scientist to join the biotechnology team at its Group Research Laboratory in Cambridge.

Applicants should preferably have some research experience to M.Sc or Ph.D level, but recent graduates with an interest in biotechnology and a good first degree in a relevant subject will be considered. A broad interest in microbiology and biochemistry and the ability to adapt to the demands of a varying work programme are essential requirements.

The work will involve both pure and applied research into biotechnological areas of interest to agriculture and the food industry, such as fermentation processes, novel secondary metabolites and the application of food enzymes.

Although the post is based in Cambridge, the successful applicant will be expected to interact with our genetic engineers working at the Leicester Biocentre.

Salary and benefits are those expected of a major international company.

Please apply with a detailed CV, SAF or write or telephone for an application form to: Anastasia Kelly, Dalgety U.K. Limited, Group Research Laboratory, Station Road, Cambridge. Tel: (0223) 359181.

(2301)A

SUNDERLAND POLYTECHNIC

NAB SELECTIVE INITIATIVE

Sunderland Polytechnic has secured a high level of funding under the NAB Selective Initiatives for developments in areas of excellence. Many special appointments are to be made on an annually renewable basis for three years, including:

FACULTY OF PHARMACEUTICAL SCIENCES
DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

POST DOCTORAL FELLOW £8,595—£10,059

RESEARCH ASSISTANT £6,060—£6,843 (two posts)

One post doctoral fellowship and two research assistantships are available for the following projects:

1. Synthesis of analogues of bacterial cellwall peptides and study of immunomodulatory properties.
2. Production of autoantibodies is psychiatric diseases, particularly with respect to schizophrenia and autism.
3. Design and synthesis of novel compounds based on peptides which are mediators in the immune response.

Applicants should possess a good honours degree in chemistry, pharmarcy or a related discipline and, for post-doctoral work, recent Ph.D.

DEPARTMENT OF PHARMACOLOGY

RESEARCH ASSISTANT £6,060—£6,843 (three posts)

The above posts are available for research concerned with ion movements and pharmacological actions, particularly in immunopharmacology cardivascular pharmacology and brain pharmacology. Applicants should possess a good honours degree in pharmacology.

NORTH EAST BIOTECHNOLOGY CENTRE

RESEARCH ASSISTANTS In Biotechnology

£6,060—£6,843 (three posts)

Applicants should hold a first or second class honours degree in a relevant biological science. One post involves research into the molecular analysis of transposable genetic elements in plants. The other would suit an individual with a microbiological/biochemical background and will involve the investigation of a microbial biotransformation system of potential commercial importance.

Research Assistant are normally expected to register for M.Phil/Ph.D.

Application forms and further particulars (to be returned by 17th March, 1987) are available from the Personnel Officer, Sunderland Polytechnic, Langham Tower, Ryhope Road, Sunderland, SR2 7EE or telephone (091) 5676231 Ext. 11. An equal opportunities employer. (2292)A

PHILIPPS-UNIVERSITÄT MARBURG

Im **Fachbereich Biologie** ist eine

Professur (C3) für Genetik

zu besetzen.

Bewerber(innen) sollen eine molekulargenetische Forschungsrichtung vertreten. Sie sollen bereit sein, mit der am Fachbereich vorhandenen molekulargenetischen Gruppe, die sich mit Genetik von Mikroorganismen beschäftigt, zusammen-zuarbeiten und sich am Unterricht des Fachs Genetik im Grund-und Hauptstudium zu beteiligen.

Für die Ernennung zum Professor sind grundsätzlich pädagogische Eignung und — als Nachweis der Befähigung zu wissenschaftlicher Arbeit — neben der Promotion die Habilitation oder gleichwertige wissenschaftliche Leistungen erforderlich. Im Übrigen gelten die Einstellungsvoraus-setzungen des § 39a HUG.

Bewerbungen mit den üblichen Unterlagen sind bis zum 03. 04. 1987 zu richten an den **Präsidenten der Philipps-Universität Marburg, Biegenstrasse 10, 3550 Marburg.** (W3857)A

HONG KONG POLYTECHNIC

Director:
Dr.John L. Clark

HEAD, Department of Applied Physics

(Re-advertisement)

The Hong Kong Polytechnic, established in 1972, is the largest institution of higher education in the territory. It offers over 200 courses up to degree and post-graduate level in a variety of subjects. The number of enrolled students is approximately 26,000 with a full-time equivalent population of around 13,000. The recently-revised academic structure has 7 divisions containing 26 teaching departments.

DEPARTMENT OF APPLIED PHYSICS

This new department has been created from the former Department of Applied Science in order to stimulate further subject development in the fields of physics and materials science, and to encourage more effective academic leadership. The Department offers a Higher Diploma course in Applied Science, has a substantial contribution to a BSc (Hons) course in Combined Studies in Mathematics and Science, and has responsibility for the teaching of physics and materials in a range of courses up to degree level in other departments. Current research interests are focussed on instrumentation, microprocessor applications, laser physics and materials science. The post of Head present a challenging opportunity to lead the new department.

Qualifications and Experience: Applicants should have appropriate academic and/or professional qualifications together with successful, relevant experience in, for example, tertiary education, industry, business, the public sector, advanced teaching, research or consultancy. The successful candidate will also be required to demonstrate the personal qualities necessary to lead the department in its diverse functions.

Salary and Conditions of Service: Not less than HK$372,600 p.a. + 25% gratuity (£1 = HK$11.85 on 23.2.87)

The initial appointment will be made on a fixed term contract of four years at the end of which a gratuity equal to 25% of salary earned over the whole contract period will be payable. Continuation thereafter is subject to mutual agreement. Other benefits include subsidised housing, leave, passages, medical and dental benefits, and children's education allowance.

Application: Application forms and detailed further information are obtainable from the Association of Commonwealth Universities (Appts), John Foster House, 36 Gordon Square, London WC1H 0PF. Completed application forms should be returned by **27 March 1987** with the first copy to be sent directly to the General Secretary, Hong Kong Polytechnic, Hung Hom, Kowloon, Hong Kong, and the second copy to the Association of Commonwealth Universities.

Interviews: The Director will be visiting the UK in early April and would wish to see selected candidates during the week commencing 6 April. Notification will be made by the Association of Commonwealth Universities. (W3879)A

UNIVERSITY OF DUBLIN
TRINITY COLLEGE

CHAIR OF PHARMACEUTICS

Applications are invited for this new appointment within the Department of Pharmaceutics. Applicants should have an established record in research and teaching: a pharmaceutical qualification would be advantageous but not essential.

Further particulars and conditions of appointment may be obtained from

G. H. H. Giltrap
Secretary to the College
West Theatre
Trinity College
Dublin 2

to whom formal application may be made, preferably not later than *18 April 1987*. (2244)A

THE NEW SOUTH WALES
INSTITUTE OF TECHNOLOGY
SYDNEY AUSTRALIA

Equal Employment Opportunity is Institute Policy

School of Biological and Biomedical Sciences

LECTURER IN URBAN HORTICULTURE

(Re-advertised)

A new part-time undergraduate degree programme in Urban Horticulture was offered for the first time in 1986, and it encompasses nursery production and management; the selection, micropropagation and breeding of new cultivars, including Australian natives; open space establishment, maintenance and management; and revegetation strategies. The programme has available to it the greenhouse and associated facilities of the Ryde School of Horticulture.

Applications are invited to assist in the development and presentation of the programme. Applicants should possess appropriate tertiary qualifications, preferably at the doctoral level. It is expected that applicants will have experience in some branch of horticulture, and a research interest in a relevant field such as tissue culture or plant pathology.

Salary range $A27,859-$A36,600 p.a.

Further information may be obtained by writing to Dr. Burchett, Head of Department at the following address:

Applications should include a detailed curriculum vitae and the names and address of three referees from whom confidential reports may be obtained. In reply please quote Reference No. K150/34 and forward by April 10, 1987.

The Director,
NSW Govt. Offices,
66 Strand LONDON. WC2N SL2.

(W3854)A

A POSTDOCTORAL POSITION

is available for a biologist/cell biologist to study heart-derived cells in a grant project limited to 4 years. A background in Molecular Biology is desirable. Salary according to experience between DM 46,450.00 and DM 58,540.00 per annum.

Applications should be sent to: **Professor Dr W. Schaper, Max-Planck-Institute, Department of Experimental Cardiology, Benekestrasse 2, D-6350 Bad Nauheim, Federal Republic of Germany.** (W3870)A

NATIONAL INSTITUTE FOR MEDICAL RESEARCH LABORATORY OF MOLECULAR EMBRYOLOGY

Applications are invited for a three year postdoctoral position in the Laboratory of Molecular Embryology, headed by Dr Brigid Hogan, for studies on gene expression during mouse embryogenesis. Specific projects are (i) identification of factors controlling the transcription of cloned developmentally regulated genes, (ii) analysis of spatial patterns of gene expression using *in situ* hybridization, and (iii) introduction of developmental genes into transgenic mice. Experience in molecular biological techniques an advantage.

The successful candidate will be supported by the excellent facilities available at the Institute and a salary in the range of £9,495 - £11,790 pa (UGC Pay Award Pending) plus £1,393 London Allowance and superannuation provision.

Please send applications including full curriculum vitae and the names of two referees to Mr C R Russell, Head of Administration, NIMR, The Ridgeway, Mill Hill, London NW7 1AA by Friday 27 March 1987 quoting reference ME/871.

An Equal Opportunities Employer. (2281)A

UNIVERSITY COLLEGE LONDON

MULLARD SPACE SCIENCE LABORATORY

Holmbury St Mary
Dorking, Surrey RH5 6NT, UK

RESEARCH IN SPACE PLASMA PHYSICS

Posts are available from 1 July for postdoctoral research assistants to work on the analysis of data from the AMPTE, Giotto, Meteosat and GEOS missions on topics such as Earth and cometary bow shocks, reconnection, artificial plasma clouds and/or on the development of instrumentation for future space missions (e.g. CRRES, CRAF, CENTAUR, GGS). For further information contact Dr A. D. Johnstone (0306-70-292).

RESEARCH IN SOLAR PHYSICS

Postdoctoral research assistant required now to work on the analysis of data from the operation of a solar X-ray spectrometer on the NASA Solar Maximum Mission. The successful candidate will also have some involvement in preparation for the flight of a high sensitivity spectrometer on the Japanese Solar-A mission.

Starting salaries in the range £8,020—£10,868 (1985 levels-increase imminent) according to age and experience. Send c.v. and the names of two academic referees to Professor J. L. Culhane, FRS, Director, MSSL. (2274)A

UNIVERSITY OF HONG KONG

LECTURER IN ZOOLOGY

(Re-advertisement)

Applications are invited for a Lectureship in Zoology. Preference will be given to applicants interested in molecular biology, with experience in immunology or developmental biology. The appointee will be expected to share in the teaching of the general area of zoology as well as developing courses in his speciality.

Annual salary (superannuable) is on an 11-point scale: HK$176,880—295,680 (approx. £14,860—24,850; sterling equivalent as at February 18, 1987). Starting salary will depend on qualifications and experience. At current rates, salaries tax will not exceed 17% of gross income. Housing benefits at a rental of 7½% of salary, children's education allowances, leave, and medical benefits are provided.

Further particulars and application forms may be obtained from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Appointments Unit, Registry, University of Hong Kong, Hong Kong. Closes: 1 May 1987.

(W3874)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

POSTDOCTORAL SENI RESEARCH ASSISTA

Applications are invited fo
above post in the Departme
Pharmacology and Therape
The project is to isolate and
tify the inhibitors responsibl
the drug binding defect of ura
plasma. The project will in
high-performance liquid chr
tography (HPLC), measure
of binding by equilibrium di
and also the identificatio
organic compounds by the u
physical methods such as
spectrometry. Applicants sh
have a strong background in c
istry and either possess or be a
to complete their PhD. Ex
ence of chromatography an
identification of organic
pounds would be an advantag

The post is funded by
Medical Research Council fo
year at a salary within the r
£8,020—£9,495 per annum (u
review).

Informal enquiries may be r
to Dr W.E. Lindup, Tel. 051
6022 Ext. 2935.

Applications, together witl
names of three referees shoul
received not later than 5th
1987 by the Registrar, The Un
sity, P.O. Box 147, Liverpool,
3BX from whom further par
lars may be obtained. Quote
RV/370/N. (2251)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF MATERIA SCIENCE AND ENGINEERIN

POSTDOCTORAL SENI RESEARCH ASSISTAN

Applications are invited for a P
doctoral Senior Research As
ant to work with Professor C
Humphreys on the mechani
and applications of a novel met
of electron beam lithography
nanometre scale. The research
use a new VG501 Field Emis
Gun Scanning Transmission E
tron Microscope (FEGSTEM
be delivered to the Departm
shortly. Applicants should h
or expect to obtain soon, a P
Experience of using a FEGST
on physics or materials scie
problems is essential. The pro
is funded for three years by
SERC, as part of its Low Dim
sional Structures (LDS)
gramme. Initial salary will be w
in the range £8,020—£9,000
annum (under review).

Applications forms and furt
particulars may be obtained fr
The Registrar, The Univers
P.O. Box 147, Liverpool,
3BX, by whom completed fo
should be received not later t
23 March 1987. Quote ref.
371/N

Coleg Prifysgol Cymru
berystwyth
ıe University College of Wales

DEPARTMENT OF AGRICULTURAL BOTANY

RESEARCH ASSISTANT

Research Assistant is required to work
the genetic control of B-Chromosome
nsmission rates in natural plant popu-
ions. The project is under the direction
Dr R. N. Jones and is funded by the
:RC for three years starting October
87. Salary will be within the range
,055 to £8,020 per annum. Candidates
ll require an honours degree in Bio-
gical Science with training in cyto-
netics, and an aptitude for Computer
odelling.

plication forms and further particulars
n be obtained from the Staffing Officer,
ıe University College of Wales, Old
llege, King Street, Aberystwyth SY23
X, (Tel. No. 0970 3177 Ext. 207).
osing date for applications, 30 April
87. (2248)A

SSOCIATE SCIENTISTS:

onduct scientific research to
escribe the pharmacology and
xicology of potential protein-
rug candidates; includes defin-
g the bio-disposition, meta-
olism of protein-drug candi-
ates and conducting biochem-
al toxicology studies as appro-
riate; provide toxicological
xpertise and advice on research
ecessary for registration of
rotein-drug candidates. PhD or
quivalent Pharmacology; 2 yrs.
xp. or 2 yrs. rsch exp. Pharma-
ology/toxicology. Rsch. exp.
ıust include drug metabolism,
harmacokinetics and biochem-
al toxicology. $46,000/year.

ob site: So. San Francisco, CA.
lip ad and send w/résumé to
ob Order #MA 0904, P.O. Box
560, Sacramento, CA 95823–
560 no later than March 20,
987. (NW592)A

LONZA

We are a versatile and internationally recognized chemical concern with worldwide contacts. Our biotechnological department needs a

microbiologist

with experience in the selection and characterization of microorganisms.

We expect:

- A completed university education (or equivalent), preferably with research experience.
- Initiative and a willingness to work hard
- The ability to work with an interdisciplinary and performance orientated team

We offer:

- The opportunity to carry out varied and independent research
- Modern employment condition
- A favourable benefits package

If you have the necessary qualifications we would like to get to know you. Please send us your written application.

LONZA AG, Walliser Werke
Stabsstelle Personal
CH-3930 Visp
✆ Switzerland / 028 / 48 53 49 (W3856)A

ASSISTANT PROFESSOR AND ASSISTANT NEMATOLOGIST:

Available July 1, 1987, an 11-month, tenure-track position as Assistant Professor and Assistant Nematologist. Research, teaching, and student advising responsibilities in the areas of host-parasite relations with plant-parasitic nematodes at the cellular, tissue, and whole-plant level, including the understanding of the role of nematodes in disease complexes and the development of management strategies for plant-parasitic nematodes in crop systems. A Ph.D. and experience in host-parasite relations, plant pathology, entomology, and nematology, are requried, with a record of research productivity.

Application deadline: June 1, 1987. Send curriculum vitae, statement of research and teaching interests relative to applying expertise in Nematology, official undergraduate and graduate transcripts, reprints of relevant publications and submitted manuscripts, names and addresses of at least three references to: **Howard Ferris, Search Committee Chairman, Division of Nematology, University of California, Davis, California 95616.**

The University of California is an Affirmative Action/Equal Opportunity Employer. (NW594)A

DIVISION CHIEF

The Department of Pediatrics of the University of Florida, College of Medicine, is seeking applications for the position of

CHIEF OF THE DIVISION OF HUMAN GENETICS.

This is a senior faculty position and appointment is anticipated at the associate or full professor level. The human genetics programs represent an integrated unit that provides clinical genetic services to northern Florida. Members of the Division conduct clinical and basic scientific research and are responsible for teaching genetics to medical and graduate students, residents, and fellows. Candidates should have an M.D. degree and should provide evidence of demonstrated leadership abilities in teaching, research, and clinical activities. A strong background in molecular genetics and its clinical application is highly desired. Salary is negotiable. Applications including curriculum vitae should be sent to: **Lodewyk H. S. Van Mierop, M.D., Chairman, Search Committee, Department of Pediatrics, Box J-296, University of Florida Health Center, Gainsville, FL 32610.** Deadline for applications is 1 May 1987. Anticipated start date is 1 September 1987.

The University of Florida is an Equal Opportunity/Affirmative Action Employer. (NW590)A

ETH ZÜRICH

Applications are invited for a position of

PROFESSOR OF BIOTECHNOLOGY

at the Institute of Biotechnology at the
ETH Zurich

Duties of the new professor will include teaching at undergraduate and graduate levels. In addition to the existing curriculum new courses may be introduced. In research cooperation with one or more of the existing groups working in gene technology, cell biology, process engineering and plant molecular biology is expected.

The successful candidate will have teaching experience and an outstanding research record in the area of gene expression using animal cell systems; experience with transgenic animals is desirable.

Applications with curriculum vitae and list of publications (no publications) should be directed before April 30, 1987, to the President of the ETH Zurich, ETH-Zentrum, 8092 Zurich, Switzerland. (W3866)A

VISITING INVESTIGATOR

A vacancy exists for an **IMMUNOLOGIST** wishing to spend one or more years in a laboratory investigating the regulation of anti-tumor immunity as a model of the immune response to replicating antigens. Applications from MD's interested in gaining research experience, or established investigators wishing to spend a sabbatical year will be considered. The salary will be $25,000 plus health insurance, and Institute housing is available.

The Trudeau Institute is a private, not-for-profit, basic medical research institution located on the shore of Saranac Lake, and near Lake Placid, NY.

Send curriculum vitae and names of three references to: **Dr Robert J. North, Director, Trudeau Institute, Inc., P.O. Box 59, Saranac Lake, NY 12983.** (NW579)A

THE CENTERS FOR DISEASE CONTROL (CDC)

is seeking an accomplished PhD

VIRAL IMMUNOLOGIST

to conduct basic and clincial research on the human immune response to human immunodeficiency virus (HIV). Experience in virus-specific proliferative and cytotoxic assays, immune cell phenotyping and separation, immunogenetics, cell culture, virus propagation and virus detection assays is required. Position is in the Immunology Branch, Division of Host Factors, CDC, which consists of 12 professional and technical people conducting investigations in various aspects of the immunology of AIDS. In addition, there are more than 100 people at the CDC conducting AIDS investigations providing additional resources for interaction and collaboration. Salary: $32,567—$38,727. Send inquiries to: **Louise Griggs, Personnel Management Specialist, CDC, Bldg. 1, Room 1042, Atlanta, Georgia 30333.**

CDC is an equal opportunity employer and has a smoke-free work environment. (NW596)A

UNIVERSITY OF HONG KONG

SENIOR HOSPITAL IMMUNOLOGIST

Applications are invited for the post of Senior Hospital Immunologist for the Tissue Typing Laboratory in the Immunology Section of the Department of Pathology's Hospital Pathology Services, tenable from January 1, 1988. Applicants should possess a higher degree in a subject related to clinical immunology and have substantial experience in tissue typing. Less qualified/experienced candidates may be considered for appointment as Hospital Immunologist. The appointee will be required to run the Tissue Typing Laboratory within the Hospital Pathology Services; this Laboratory provides a centralized service for the whole of Hong Kong.

Annual salaries (superannuable) are on the scales: Senior Hospital Immunologist: HK $274,980—369,360 (9 points) (approx. £23,110—31,040), Hospital Immunologist: HK$176,880—295,680 (11 points) (approx. £14,860—24,850); (Sterling equivalent as at February 16, 1987). Starting salary will depend on qualifications and experience. At current rates, salaries tax will not exceed 16.5% of gross income. Housing benefits at a rental of 7½% of salary, children's education allowances, leave, and medical benefits are provided.

Further particulars and application forms may be obtained from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Appointments Unit, Registry, University of Hong Kong, Hong Kong. Closes: 18 April 1987. (W3876)A

THE QUEEN'S UNIVERSITY OF BELFAST

DEPARTMENT OF PHYSIOLOGY

RESEARCH ASSISTANT

Available for one year in the first instance to work on lymphatic physiology in collaboration with Professor I. C. Roddie, this post, funded by the British Heart Foundation would be suitable for pre- or postdoctoral graduates in Biology or Medicine.

Salary: £7,056—£9,495 per annum, initial placing depending on age, qualifications and experience.

Applicants, quoting ref. 87/D, should submit a curriculum vitae including the names and addresses of two referees to the Personnel Officer, The Queen's University of Belfast, Belfast BT7 1NN, Northern Ireland. Closing date: 31

THE UNIVERSITY OF NEW SOUTH WALES

Sydney, Australia

PROFESSOR OF PHYSI

Applications are invited appointment to a Chair of Ph in the area of Experime Physics. The appointment wi available from January 1988.

Applications are invited f physicists with substantial search experience, preferably lated to the present activities o school. Preference will also given to applicants whose fi are relevant to areas of mo technology at present existin Australia or likely to be develo in the near future. The new pr sor would be expected to serv Head of Department or Hea School for a term or terms i requested.

The School of Physics compr five departments: Theore Physics, Biophysics, Conder Matter Physics, Astrophysic Optics and Applied Physics, has the largest enrolment graduate students of any Ph School in Australia.

Enquiries regarding the C may be addressed to Professo St. C. Black, Acting Dean of Faculty of Science, or from present Head of School, Assoc Professor J. C. Kelly, Unive of New South Wales, P.O. Bo Kensington, New South W 2033, Australia, from whom ther information may be obtai regarding specific areas of search interest in the departm of the School.

Salary: $A58,348 per annum

Subject to consent by the versity, professors may undert a limited amount of higher sultative work.

The University reserves right to fill any Chair by invitati

For full information about ditions of appointment method of application write to Secretary General, Associa of Commonwealth Universi (Appts), 36 Gordon Squ London WC1H 0PF.

Applications close: 15 1987.

Equality of employment op tunity is University policy.

(W3859)

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nature

the widest
international
selection of jobs
in science
— EVERY WEEK

UNIVERSITY OF AUCKLAND
New Zealand

LECTURESHIP/ ENIOR LECTURESHIP IN BOTANY

licants for this post in the elopmental Botany Section of Department of Botany should a PhD degree and will be exed to participate fully in both ning and research. The Dement has established vigorous arch programmes on the iology, development and tics of 'sub-tropical' fruits and erence may be given to those experience in developmental/ physiology. Applicants ld have a proven ability to funds, and to organise and ct relevant research promes.

ommencing salary will be rmined in accordance with ifications and experience in the following ranges: Lecr NZ$30,500 to $35,000, ior Lecturer NZ$37,000— ,000 per annum.

onditions of Appointment and hod of Application are available from the Secretary General, ociation of Commonwealth versities (Appts), 36 Gordon are, London WC1H 0PF; or n the Assistant Registrar ademic Appointments), University of Auckland, Private Bag, kland, New Zealand, to whom lications in accordance with Method of Application, should orwarded as soon as possible, not later than 13 April 1987.

(W3860)A

UNIVERSITY OF ABERDEEN
EPARTMENT OF PATHOLOGY

IMMUNOPATHOLOGY LABORATORY
POSTDOCTORAL RESEARCH FELLOW

uired to join a multi-disciplry team investigating the ieration of immune responses human immunodeficiency virus IV) infected cells. Experience tissue culture, hybridoma production, maintenance of cell lines, tein analysis techniques — S — Page, western blotting, is ential. The appointment is ded by a Medical Research uncil special project grant for 3 irs.

Starting salary £9,000 p.a. on Scale for Research and Analous Staff (under review).

Further particulars and application forms available from The Personnel Office, The University, gent Walk, Aberdeen, AB9 X, with whom applications (2 ies) should be lodged by 31 rch 1987. (Ref. WS/018).

UNIVERSITY OF ABERDEEN
DEPARTMENT OF MEDICINE AND THERAPEUTICS

POSTDOCTORAL RESEARCH FELLOWS
(2 POSTS)

1) to work in Haematology Unit using mini satellite probes to assess DNA damage in patients with lymphoma receiving cytotoxic chemotherapy and the relation of such damage to induction of secondary malignancies. The appointment is for 3 years. (Ref. WD/051).

2) to work in Endocrinology Unit to study the synthesis, structure and immunochemistry of thyroglobulin in relation to thyroid disease. The appointment is for 5 years. (Ref. WD/052).

DEPARTMENT OF GENETICS AND MICROBIOLOGY

RESEARCH ASSISTANT

to work on a project to establish genetic linkage between X-linked peroneal muscular atrophy and several available cDNA probes. The appointment is for 2 years. (Ref. WD/053).

Salary within the range £8,020—£12,780 p.a. (with appropriate placing) on 1A Scale for the Research Fellow posts and up to £7,530 p.a. on the 1B Scale for the Research Assistant post. (Scales under review).

Further particulars and application forms from the Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX, with whom applications (2 copies) should be lodged by 3 April 1987 quoting the appropriate ref. no.

(2255)A

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF PHARMACOLOGY

POSTDOCTORAL RESEARCH ASSOCIATE IN CELL BIOLOGY

Applications are invited for the above post to work on an MRC-funded project to apply a newly-developed cell-free vesicle fusion system to a study of the biogenesis of the plasma membrane in eukaryotic cells. Applicants should ideally be familiar with cell culture, cell fractionation and basic immunological procedures. The post is available for three years from 1 April 1987. The maximum starting salary will be £9,495, depending on age.

Further information may be obtained from Dr J. M. Edwardson, Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 2QD (0223-336932), to whom applications, together with c.v. and the names of two referees, should be sent, as soon as possible.

INSTITUTE OF
CLINICAL PHARMACOLOGY (Ireland) Ltd.
P.O. BOX 469, DUBLIN 8.

CLINICAL PROJECT MANAGERS

ICP is an international clinical research management and consultancy organisation whose clients include major pharmaceutical companies worldwide.

ICP is seeking to appoint Clinical Project Managers. Ideal candidates should have a medical/science qualification with proven ability to establish and conduct clinical trials to meet international regulatory requirements.

Terms and conditions of employment are negotiable and will be commensurate with qualifications and experience. The successful applicant will be based in Bath but must be prepared to travel within Europe. Preference will be given to applicants with language skills and computer literacy.

All applications will be treated in the strictest confidence and should be addressed to:

Dr. S. Jevons,
Managing Director,
ICP Ltd.,
15 Gay Street,
Bath BA1 2PH, England.

(2297)A

ICP

POSTDOCTORAL RESEARCH ASSOCIATE

position available July 1, 1987 in a laboratory specializing in comparative endocrinology. Research will involve the production and purification of monoclonal antibodies to biologically active neuropeptides. A working familiarity with radioimmunoassays, HPLC, and monoclonal technology is essential. The positions requires teaching of human gross anatomy to Medical Students and prior experience in gross anatomy is preferred. Send applications to **Dr Noelle Granger, Department of Anatomy, UNC at Chapel Hill, 350 Swing Bldg. 217H, Chapel Hill, N.C. 27514.**

The University of North Carolina at Chapel Hill is an Equal Opportunity/Affirmative Action Employer. (NW584)A

— POSITION OPEN —
CELLULAR NEUROBIOLOGIST/ DEVELOPMENTAL BIOLOGIST

The Department of Biological Sciences, Stanford University, has several openings for tenure-track faculty positions in the areas of cellular neurobiology and developmental biology. Applicants at the assistant professor level are preferred, but the Department will also consider appropriate candidates at a more senior level for one of these positions. Applicants should send their curriculum vitae, a statement of research accomplishments and anticipated research directions, and the names of three appropriate references to: **Chairperson, Neurobiology Search Committee, Department of Biological Sciences, Stanford, CA 94305-5020.** Applications from women and minority candidates are especially welcome. *Stanford University is an equal opportunity employer.*

(NW580)A

Royal Holloway and Bedford New College

UNIVERSITY OF LONDON
EGHAM HILL·EGHAM·SURREY·TW20 0EX

DEPARTMENT OF BIOCHEMISTRY

Postdoctoral Research Assistant

Applications are invited for a postdoctoral Research Assistant starting as soon as possible to work with Dr J R Bowyer on herbicide interactions with chloroplast Photosystem II. The project is funded by Shell Research Ltd and will run for an initial period of 2 years.

Applicants should have a PhD in biochemistry with experience in protein chemistry including peptide fingerprinting and analysis, and should preferably have an interest in photosynthetic electron transport.

The salary will be on the RA IA Scale, depending on age and experience, and will include London allowance of £1393 per year.

Application forms are available from Miss K Clarke, Personnel Office. Informal enquiries can be made to Dr J R Bowyer Biochemistry Department on 0784 34455 extension 3803. (2270)A

POSTDOCTORAL POSITIONS

available immediately for three neurobiologists interested in development and regeneration. Research involves intermediate filament and extracellular matrix proteins. Experience in protein chemistry, tissue culture, or electron microscopy desirable. Training opportunities in immunohistology.

Salary range: $18,000 to $26,000 depending on experience. Please send curriculum vitae to: **Dr. Amico Bignami, Department of Pathology, Harvard Medical School, and Spinal Cord Injury Research, West Roxbury, VA Medical Center, Boston, MA 02132.** (NW598)A

PHYSIOLOGY
ASSISTANT RESEARCH

Minimum three years experience Physiologist or Biophysicist with PhD degree related field to conduct biophysical and electrophysiological investigations into the mechanism of ion transport in epithelial tissues. Must have understanding of mathematics, electronics and physiology and be thoroughly familiar in applying patch clamp techniques and fluctuation analysis to study ion channels. Should be familiar with computers and be able to interface them to laboratory instruments and to write programs for data acquisition and analysis.

Salary $34,600 yr.

Job site and interviews: Los Angeles, California. Send this Ad. and your resume to job # AW 1576, P.O. Box 9560, Sacremento, California 95823-0560 not later than March 20, 1987. (NW605)A

POSTDOCTORAL TRAINING PROGRAM IN THE CELLULAR/MOLECULAR BIOLOGY OF AGING

Positions are available for postdoctoral trainees (PhD and/or MD) to obtain two years of specialized training in the methods and models of research on aging. This program is directed by a consortium which includes the University of Pennsylvania/Wistar Institute (Drs V Cristofalo, D Kritchevsky, E Levine), Temple University (Drs R Baserga, N Duker, A Schwartz), the Medical College of Pennsylvania (Dr J Roberts) and Merck Sharp and Dohme Research Laboratories (Drs M Bradley and W Nichols). Research emphases include: cell cycle kinetics/dynamics; growth factor regulatory mechanisms; growth regulatory genes; cellular transformation and immortalization; regulation of gene expression; DNA repair; cytogenetics; nutrition; vascular cell physiology; cardiovascular physiology/pharmacology; and DHEA action in aging and cancer.

Applicants must meet NRSA United States citizenship or residency requirements. Address applications (curriculum vitae, three letters of reference and graduate school transcript) to: Vincent J Cristofalo, PhD, Director, University of Pennsylvania, Center for the Study of Aging, 3906 Spruce Street, Philadelphia, PA 19104. (NW570)A

PLANT BREEDING INSTITUTE
HYBRID WHEAT PRODUCTION AND TRIALS OFFICER

required for a period of two years funded by the Agricultural and Food Research Council to work on hybrid wheat.

The appointee will join a small team investigating the feasibility of breeding hybrid what varieties for the UK, and the work will include the submission of hybrids for National List Trials.

Qualifications: Degree in Agricultural Botany, Applied Biology or relevant plant science preferred but not essential. Post-qualifying experience and conduct of yield trials in a plant breeding or other field assessment programme would be particularly useful, as would basic computer skills.

Appointment will be at Scientific Officer (salary £6,711 to £9,225 per annum). Non-contributory pension scheme.

Applications with c.v. and names and addresses of two referees should be sent to the Personnel Officer, Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, quoting reference CER/270, by 31st March 1987.

The Institute is an Equal Opportunities Employer. (2286)A

THE UNIVERSITY OF SYDNEY
CHAIR OF PATHOLOGY
Reference No. 8/01

Applications are invited for a Chair of Pathology. Applicants need not be medical graduates but must have extensive experience in experimental pathology and in the field of cell biology.

Salary will be at the rate of $A58,348 per annum and there is provision for a paraclinical loading of $A6,964 per annum where appropriate.

The University reserves the right not to proceed with any appointment for financial or other reasons.

Intending applicants should obtain a detailed statement of information concerning the Chair from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications, in duplicate, quoting reference no., including curriculum vitae, list of publications and the names and addresses of three referees by 29 May 1987 to the Registrar, University of Sydney, NSW 2006, Australia. (W3858)A

UNIVERSITY OF WAL
COLLEGE OF MEDICI

DEPARTMENT OF MEDICA
BIOCHEMISTRY

A RESEARCH POST

has become available for wor
a study of reactive oxygen me
olite production by neutrop
and their role in inflammatory
immune-based disease. The p
funded by a project grant from
Medical Research Council to
A. K. Campbell, and involves
development of chemi- and
luminescent indicators by de
ing molecular events in s
cells. The project interacts cl
with others utilising image a
sis, cloning techniques and fl
escence activated cell sorting
PhD or other higher degree w
be an advantage but not essen

Salary within the range £7,0
£9,880 per annum on the scales
University Research and An
gous Staff (substantial pay
pending); starting point de
dent upon qualifications and
perience.

Further particulars avail
from the Registrar and Secre
(Personnel Office), Universit
Wales College of Medicine, H
Park, Cardiff CF4 4XN quo
ref. No. C38/4/59 (tel. No. 0
755944 ext. 2296).

More detailed information f
Dr A. Campbell (ext. 2803).
(2269)

Postdoctoral Fellow. Appl
tions are invited for a Postdoct
Fellow interested in utilizing
techniques of molecular biol
to study the synthesis and st
ture of intestinal mucus gly
proteins in normal and pat
logical conditions (e.g. cy
fibrosis and inflammatory bo
disease). Prior experience in
field of molecular biology
highly desirable but knowledg
glycoprotein biochemistry is
essential.
Starting salary will be appr
mately $20,000 (Canadian)
cording to qualifications
experience.
In accordance with Canad
immigration requirements,
ority will be given to Canad
citizens and permanent reside
of Canada.
Enquiries and applications,
cluding curriculum vitae and
names and addresses of th
referees, should be sent to: **Dr
Mantle, Department of Medi
Biochemistry, The University
Calgary, Health Sciences Cen
3330 Hospital Drive N.W.,
gary, Alberta T2N 4N1.**
(NW576)

UNIVERSITY OF LEICESTER

)EPARTMENT OF PHYSICS

lications are invited for two
nt positions for Research
ciates to work in the space
umentation programme of the
ersity of Leicester's X-Ray
onomy Group.

th the ROSAT XUV Wide
l Camera now expected to be
ched in 1989, an experimental
icist with interests in X-Ray or
astronomy is required, a) to
icipate in the calibration pro-
me of the flight instrument,
b) to work on scientific plan-
or software for the operations
data analysis phases.

new instrument concept in-
ing novel uses of charge
led devices (CCD's) is being
loped for approved European
e Agency scientific missions.
experimental physicist is re-
ed to join the development
to work on the design and
uation of the prototype in-
ment.

oth positions are for 3 year
intments on the postdoctoral
earch Associate RA1A scale.
ry, depending on age and ex-
ence, will be in the range
03 to £12,603 p.a., (£9,865 to
,364 after March 1st, 1988).
ary scales subject to confirm-
n).

pplications should be sent to
fessor K. A. Pounds, CBE,
, Department of Physics, Uni-
ity of Leicester, together with
and the names and addresses
wo referees. The closing date is
rch 31st, 1987. (2280)A

ARIBBEAN REGIONAL DRUG TESTING LABORATORY

MICROBIOLOGIST POSITION

e Caribbean Regional Drug
ting Laboratory in Kingston,
naica invites applications to fill
post of Microbiologist.

)uties: Testing and evaluating
microbiological quality of
rmaceutical products, includ-
the development and applica-
n of techniques for the assaying
antimicrobials.

)ualifications: MSc or PhD
gree in Microbiology. Experi-
e in the analysis quality control
pharmaceutical products pre-
red.

Applicants should submit curri-
um vitae with names and ad-
sses of three references to: The
rector, Caribbean Regional
ug Testing Laboratory, c/o
vernment Chemist's Depart-
nt, Hope, Kingston, 6, Jama-
, WT.

The closing date for the receipt
applications is 13th March,
27 (W2869)A

HIGHER SCIENTIFIC OFFICER

ORNITHOLOGIST

Reference No: HE2 36 75

Period appointment, initially for 3 years, with a possibility of extension up to 5 years. The post will be based at Peterborough.

NCC has a responsibility to advise government as to the fulfilling of its international commitments to conservation, including those under the EC Directive on the conservation of wild birds. An ornithologist is required to collate and review existing data on the extent to which site-safeguard meets these commitments, and to assist on the development of conservation measures for wide-ranging species.

QUALIFICATIONS: A degree or equivalent in a biological subject plus at least 2 years relevant postgraduate research experience. Wide ornithological knowledge, writing experience and the ability to collate diverse data are essential. Computing and statistical expertise and a knowledge of land-use practices would be advantageous.

SALARY: Between £8,405 and £11,323 dependant upon qualifications and experience. The appointment is pensionable.

Application forms and further details are available from: Personnel 1, The Nature Conservancy Council, Northminster House, Peterborough PE1 1UA. The closing date for completed applications is 27 March 1987. Interviews will be held in late April. NCC is an equal opportunities employer. (2261)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL

(University of London)

POSTDOCTORAL MOLECULAR MICROBIOLOGIST

Applications are invited for this post to join an active research group involved in the application of recombinant DNA techniques to the study of Crohn's disease associated organisms. The research would involve the development and use of DNA and RNA probes for the sensitive detection and characterisation of mycobacteria. The post, which is funded by the Wellcome Trust, is available for three years with a starting salary in range 1A for University research staff of up to £11,672 inclusive of London Allowance (salary under review).

Position on salary scale determined by reference to experience and qualifications. Applications with the names of two referees should be sent to Professor J. Hermon-Taylor, Department of Surgery, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, 01-672 1255 ext 4716 (2253)A

FACULTY POSITION IN MOLECULAR GENETICS

University of Southern California School of Medicine

The Department of Biochemistry and the Comprehensive Cancer Center, University of Southern California School of Medicine invite applications for tenure-track faculty positions at the Assistant or Associate Professor level. Individuals in the area of eukaryotic molecular genetics are particularly encouraged to apply, but qualified investigators in related disciplines will also be considered.

Submit applications, including a curriculum vitae, a statement of research interests, and the names of three references, to:

Dr. Amy Lee,
Chairperson,
Molecular Genetics Search Committee,
USC Comprehensive Cancer Center,
NOR 743, 1441 Eastlake Avenue,
Los Angeles, CA 90033.

Affirmative Action/Equal Opportunity Employer.
(NW597)A

UNIVERSITY OF SOUTHAMPTON

Faculty of Medicine

CHAIR OF CHEMICAL PATHOLOGY AND HUMAN METABOLISM

Applications are invited from scientifically or medically qualified candidates for the Chair of Chemical Pathology and Human Metabolism, which will become vacant in October 1987 following the retirement of Professor B. E. Clayton. Further details may be obtained from the Secretary and Registrar, The University, Southampton, SO9 5NH, to whom applications (11 copies from applicants in the UK) should be sent before *8 May 1987*. (2263)A

UNIVERSITY OF NEW BRUNSWICK

Department of Biology

FACULTY POSITIONS

Contingent upon final budgetary approval, the Biology Department seeks to fill **three tenure-track positions: Developmental Botany, Plant Ecology,** and **Environmental Physiology/Zoology**. Applicants must have a PhD and be committed to research and teaching. The successful candidates will be expected to develop vigorous research programs, interacting with existing researchers. In addition they will participate in undergraduate and graduate teaching. The main undergraduate teaching for the three positions would include introductory botany, general ecology, and upper year courses in their specific areas of expertise. Applicants should submit a letter of application, a statement of their research interests, and a curriculum vitae with names of three potential referees to: **Dr R. T. Riding, Chairman, Department of Biology, University of New Brunswick, Bag Service No. 45111, Fredericton, N.B. E3B 6E1**. Closing date is 1 May 1987 or as soon as appointments are filled. The positions begin 1 July 1987. In accordance with Canadian Immigration requirements this advertisement is directed to Canadian citizens and permanent residents.
(NW573)A

MEDIZINISCHE HOCHSCHULE HANNOVER

The Medical School of Hannover invites applications for a

Full-Professorship in Experimental Retrovirology.

The successful applicant will be appointed Director of the Department of Virology and Immunology of the Deutsches Primatenzentrum at Göttingen. The salary is according to group C4 of the German University scale.

Applications should contain a tabular curriculum vitae, a list of publications, and a short description of scientific work both achieved and intended, and should be submitted until 31.03.87 to

Rektor
der Medizinischen Hochschule Hannover
Konstanty-Gutschow-Str. 8
D-3000 Hannover 61/West Germany

(W3865)A

POSTDOCTORATE 2D NMR—

To solve the solution structure of small (MW 6,000–10,000) proteins involved in blood coagulation phenomena. Facilities include Bruker 500, MSL 300, several 200, 300 MHz. Candidate should have experience in NMR of macromolecules. Available 1 May 1987.

Please submit curriculum vitae, reprints, and three to four references to: **Professor L.J. Berliner, Department of Chemistry, Ohio State University, 120 West 18th Avenue, Columbus, OH 43210; telephone: 614–292–0134.**

(NW595)A

Research Associate

A position is available for a lipid biochemist with two years postdoctoral experience in Differential Scanning Calorimetry, Stopped Flow Spectrophotometer, glycerolipid and prostaglandin biosynthesis. Salary $21,999. Apply by resume only to the: **MA Division of Employment Security, C. F. Hurley Building, Boston, MA 02114.** Case No. 7381.

(NW581)A

EXPERIMENT STATION OF THE HAWAIIAN SUGAR PLANTERS' ASSOCIATION

Postdoctoral Position

available immediately, to work on solubilization, separation, and characterization of enzymatically active membrane-bound proteins in vacuolar preparations from sugarcane cell cultures. Preference given to resourceful candidates with experience in enzyme kinetics, liposome reconstitution of proteins, immunological techniques, and protein purification methods. Send curriculum vitae and at least two references to: **Dr A. Maretzki, HSPA, 99-193 Aiea Heights Drive, Aiea, HI 96701.**

(NW571)A

POSTDOCTORAL OR RESEARCH ASSOCIATE POSITION

Available immediately for 2 to 3 years to participate in studies involving molecular cloning and expression of falciparum malaria genes. Should have some experience with biochemical techniques. Salary negotiable, depending on experience. **Send curriculum vitae and three letters of reference to: Dr. A.C. Vezza, Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294.** An Affirmative Action/ Equal Opportunity Employer.

(NW602)A

MEDICAL RESEARCH COUNCIL

RADIOBIOLOGY UNIT, CHILTON

MOLECULAR & CELL BIOLOGIST

Applications are invited for a Short Term Non-Clinical Post Doctoral Scientist (5 years) to study molecular aspects of DNA repair and mutagenesis, including gene cloning, in cultured mammalian cells. Practical experience in molecular genetics/recombinant DNA techniques (but not necessarily other aspects of these studies) will be an advantage.

Remuneration at an appropriate point on MRC non-clincal scales (equivalent to University non-clinical academic scales). The Unit is situated in pleasant rural surroundings on the Harwell Laboratory Site and is convenient for Oxford. Hostel accommodation may be available.

Please send applications including CV and the names of two referees to the Personnel Officer, MRC Radiobiology Unit. Chilton, Didcot, Oxon. OX11 0RD. Tel: Abingdon (0235) 834393; contact Dr John Thacker for further information. Closing date 31st March 1987.

The Council is an equal Opportunity Employer. (2295)A

LEEDS WESTERN HEALTH AUTHORITY

Clarendon Wing

RESEARCH ASSISTANT

Salary on the Basic Grade Biochemist scale £7,452—£9,360.

You are required to work on the molecular biology of abnormalities in sexual differentiation in mammals, including man in the Department of Chemical Pathology and Department of Obstetrics & Gynaecology. The appointment will be made for a fixed period of three years from 1 April 1987.

Candidates should possess an honours degree or recognised equivalent in Biochemistry, genetics or other appropriate biological science. You may be able to register for a higher degree.

Informal enquiries to Dr A.D. Stewart (0532) 432799, ext. 3927 are invited.

Application forms and further particulars may be obtained from the Personnel Officer, Clarendon Wing, Belmont Grove, Leeds LS2 9NS. Tel: (0532) 432799, ext. 3205. Closing date 19th March, 1987. (2193)A

MOLECULAR BIOLOGIST/ BIOCHEMIST/VIROLOGIST

We are looking for a Research Scientist to work on an EEC funded project concerned with the construction of linear, plasmids and artificial minichromosomes for genetic manipulation of mammalian cells. It is a collaborative project with the Universities of Rome and Tubingen, with opportunities for working vists in these laboratories.

The position, which is available immediately, would be suitable for a graduate with working experience in a research laboratory or a recently qualified doctorate. Applicants should preferably have some background in molecular biology, but enquiries from those with relevant qualifications related disciplines would be welcomed.

Higher Scientific Officer (HSO). Minimum qualifications: Good honours degree plus two years post graduate experience. Salary: £8,405–11,323. Scientific Officer (SO). Minimum qualifications: Good honours degree. Salary: £6,711–9,225.

The Institute is an equal opportunities employer. Informal enquiries can be made by telephone (0483–232441) or by letter to Dr C Bostock. To apply please send curriculum vitae and the names of two referees to:– Dr B W J Mahy (Head), Pirbright Laboratory, Institute for Animal Disease Research, Ash Road, Pirbright, Woking, Surrey GU24 0NF.

UNIVERSITY OF TASMANIA

CONTRACT LECTURESI IN BIOCHEMISTRY

(Ref No 20/87)

Applications are invited for
above post which will be avail
on a full-time contract basis
from one to five years from S
tember 1987. Candidates sh
have a higher degree in bioch
istry or a related area. Prefere
will be given to those with resea
interests in molecular biol
Candidates will be expected
have an established research b
ground and will be actively
couraged to attract research gr
and to supervise postgrad
students. Possibilities exist
either internal collaborative m
cal research within the Dep
ment of Biochemistry or exte
collaborative research with gov
ment instrumentalities of
Commonwealth and State
regional problems such as th
concerned with Antarctic
Southern Ocean studies.
present salary scale for lecture
$A27,859 × 7 increments
$A36,600 p.a. For further acade
information contact Profes
M.G. Clark (tel: 61.02.202672)

Application forms and furt
information available from
Staff Office (tel: 61.02.202013)
the Association of Commonwe
Universities (Appts), 36 Gor
Square, London WC1H 0PF.
plications quoting ref no, cl
with the Staff Officer, Univer
of Tasmania, Box 252c, G.P
Hobart, Tasmania 7001, Austra
on 15 May 1987.

The University is an equal
portunity employer. (W3878)A

Still first with the bes
in science
nature
— the world's most
prestigious weekly
journal of science
— the professionals'
choice

iculty Positions in Pharacology and Therapeutics. e University of Calgary, Department of Pharmacology and Thereutics invites applications for culty positions dedicted primar to research activities. Indiduals at any professorial rank, ho have a PhD and/or MD deee and a demonstrated ability r in dependent research in pharacology or a related discipline e encouraged to apply. Areas of rticular interest to the department are: smooth muscle funcon; molecular and cellular toxology; and transmembrane gnalling/cell regulation. Candiates must have research accomishments that would merit schorship support from granting gencies such as the Medical Rearch Council of Canada or the lberta Heritage Foundation for edical Research. Academic rank nd salary commensurate with xperience and qualifications.

accordance with Canadian imigration requirements, priority ill be given to Canadian citizens nd permanent residents of Canaa.

he deadline for receipt of appliations is May 15, 1987. Applients should apply in writing, enosing a curriculum vitae, the ames of three referees and a reearch program summary to: **Dr . D. Hollenberg, Head, Departent of Pharmacology and Therpeutics, The University of Calary, Faculty of Medicine, 3330 lospital Drive N.W., Calgary, anada T2N 4N1.**

(NW575)A

UNIVERSITY OF ABERDEEN

POSTDOCTORAL RESEARCH FELLOW

HEMIST, BIOCHEMIST OR HYSICIST with an interest in olysaccharides, carbohydrates or ree radicals is required now to oin an established research group vithin the Department of Biohemistry.

Applicants wishing to discuss he project should contact Dr rank Williamson or Dr Bill Long n 0224 480241 Extns 212/213.

Salary within the range £8,020—9,495 p.a., on 1A Scale for Reearch and Analogous Staff (under eview).

Further particulars and applicaion forms available from The ersonnel Office, The University, egent Walk, Aberdeen, AB9 FX with whom applications (2 opies) should be lodged by 27 Aarch 1987. (Ref. WS/013).

(2256)A

UNIVERSITY OF BRISTOL

DEPARTMENT OF PATHOLOGY

Applications are invited for the post of

POSTDOCTORAL RESEARCH ASSISTANT

to work on a project funded by the Wellcome Trust investigating the mechanisms, including priming signals and release of specific enzymes, by which macrophages destroy connective tissues. Candidates should have a sound knowledge of enzyme histochemistry and/or related techniques and preferably some experience in immunology and cell culture. The appointment will be for three years on Scale 1A (£8,020—£9,880 per annum with USS benefits). Further details from Professor I.A. Silver (Tel. 0272 303445).

(2249)A

POSTDOCTORAL POSITION IN MOLECULAR IMMUNOLOGY

INSTITUT JACQUES MONOD FACULTY OF SCIENCES PARIS, FRANCE

To study: the rearrangements of Ig genes in transgenic mice; the mechanism of gene conversion to generate diversity during chicken development.

PhD required, experience in molecular biology is desirable.

Interested persons should apply to: Dr J.C. Weill, Institut Jacques Monod — Tour 43 2 Place Jussieu — 75251 PARIS Cedex 05, France.

(W3867)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF MICROBIOLOGY

TEMPORARY LECTURESHIP

(One year)

Applications are invited for the post of temporary Lecturer in the Department of Microbiology. The post arises on the award of a Leave Fellowship from the Wellcome Trust to Dr J. R. Saunders. Applicants should hold, or expect shortly to obtain, a PhD and have an appropriate background in microbiology. They should be able to teach microbial genetics and pathogenicity.

The salary will be within the range £8,020—£9,495 per annum (under review) and the successful applicant should be able to start as soon as possible after 1st July 1987.

Applications, together with the names of three academic referees, should be received not later than 19 March 1987, by The Registrar, The University, P.O. Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. Quote ref. RV/372/N.

(227[illegible])A

MRC CLINICAL RESEARCH CENTRE
(Northwick Park Hospital)
Watford Road, Harrow
Middlesex, HA1 3UJ

MOLECULAR BIOLOGIST — DIVISION OF RHEUMATOLOGY

A Senior Postdoctoral Scientist is required to join an on-going project under the direction of Dr. P. Woo studying the gene expression of amyloid precursor proteins. Applicants should have postdoctoral molecular biology experience. Expertise in transcription and translation techniques is desirable. The precise area of study will depend on the background and experience of the successful candidate.
The position is tenable for three years in the first instance.
Salary scales £10,888 — £17,093 including London weighting.
Informal enquiries may be made by contacting **Dr. P. Woo 01-864 5311 Ext 2957.** ***For further details apply to the Personnel Dept Quoting ref 131/1/MB. 01-864 5311 Ext 2673.*** Closing date for completed applications 26th March 1987.
The Medical Research Council is an equal opportunity employer.

(2278)A

UNIVERSITY OF LONDON

THE DUKE OF WESTMINSTER'S CHAIR OF AGRICULTURE AT WYE COLLEGE

The Senate invite applications for the above Chair. Applicants should have leadership qualities and proven research and teaching abilities in animal or crop production or related sciences.

Applications (10 copies) should be submitted to the Teacher's Section (N), University of London, Senate House, Malet Street, London WC1E 7HU, from whom further particulars should first be obtained.

The closing date for receipt of applications is 10 April 1987.

(2265)A

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a three-year postdoctoral research post funded by the Wellcome Trust. The appointee will participate in a programme of research on the antimalarial activity of plant products and will join a team supported by the Medical Research Council and The Trust. Applicants should have expertise in biochemistry and pharmacology.

The research will be carried out at LSHTM and The School of Pharmacy under the direction of Dr D. C. Warhurst and Professor J. D. Phillipson.

Starting salary up to £11,015 plus £1,393 London Weighting on the Research and Analogous 1A scale plus membership of USS.

Applications with full curriculum vitae and the names of three referees should be sent to the Personnel Officer, LSHTM, Keppel Street, London WC2A 2AE. Closing date 31.3.87 (2283)A

INSTITUTE OF OPHTHALMOLOGY

SUB-DEPARTMENT OF VIROLOGY

A GRADUATE/ POSTDOCTORAL RESEARCH ASSISTANT/ LECTURER

with experience in the development of monoclonal antibodies is required to complement a team of micro-biologists, immunologists and clinicians, highly active in the development of advanced bacterial and viral laboratory tests and in studies of pathogenicity and host virus interactions in ocular and genital infections, and associated diseases. The post is funded for two years in the first instance, renewable subject to obtaining further funding. Salary and benefits depending on qualifications and experience. Suitably qualified candidates please send a curriculum vitae to:

Miss T. Sharp, Sub-Department of Virology, Institute of Ophthalmology, Judd Street, London, WC1H 9QS. Telephone 01-387 9621 ext. 240.

(2266)A

POSTDOCTORAL POSITION

Available to study molecular genetics of human papillomaviruses. Applicants should have experience in molecular biology, cell biology, or monoclonal antibody production to participate in research on structure and function of the viral genome to determine mechanism of cell transformation.

Send curriculum vitae, summary of research experience and names of three references to: Dr. Phoebe Mounts, Department of Immunology and Infectious Diseases, Johns Hopkins University School of Public Health, 615 North Wolfe Street, Baltimore, MD 21205.

(NW601)A

UNIVERSITY OF OXFORD

Department of Biochemistry

POST-DOCTORAL RESEARCH ASSISTANT — RS 1A and POST-GRADUATE RESEARCH ASSISTANT — RS 1B

(Ref: BR/186)

An opportunity exists for individuals who are interested in applying the techniques of multinuclear NMR and molecular genetics to investigate the relationship between energy metabolism and the synthesis of specific foreign proteins in mammalian cells grown in fermenter culture. The work, which is funded for three years under a new initiative from the SERC Biotechnology Directorate, will be carried out in the laboratories of Dr K.M. Brindle, Drs A.J. and S.M. Kingsman and Prof. G.K. Radda. We are seeking individuals with experience in mammalian cell culture and/or fermentation technology. However researchers with experience in other appropriate biochemical disciplines are encouraged to apply. The starting salaries will be within the range £8020–£9495 (RS 1A) and £7055–£8505 (RS 1B) (under review). Please submit three copies of a curriculum vitae, with the names of two referees as soon as possible to: The Administrator, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU. (2299)A

UNIVERSITY OF CAMBRIDGE

Veterinary School

RESEARCH ASSOCIATE

Applications are invited for a three-year post funded by the Equine Virology Research Foundation. The successful candidate will join an active and enthusiastic team working on the molecular biology, pathogenesis and immunology of animal herpesviruses. This project involves an investigation of the immune responses to equine herpesvirus (EHV) in SPF (EHV-free) foals and hamsters in relation to viral pathogenesis and latency. This will require a range of modern virological and immunological techniques including T-cell cloning, interferon assays and an isotype-specific ELISA for EHV.

Some experience in virology or immunology is essential. The salary range is up to £12,035 (age 29) rising to £13,080 (age 31). Applications (with curriculum vitae and the names and addresses of two referees) should be sent as soon as possible to Drs Hugh Field and Philip Duffus, University of Cambridge, Department of Clinical Veterinary Medicine, Madingley Road, Cambridge CB3 0ES (0223-337600) who can be contacted for further information. (2290)A

UNIVERSITY OF LEICESTER

Leicester Biocentre

RESEARCH TECHNICIAN

Applications are invited, from suitably qualified candidates, for the post of research technician to work on a project involving the use of tissue culture techniques to study the introduction and expression of foreign genes in plants. Experience in plant cell culture or recombinant DNA technology would be an advantage. The appointment will, in the first instance, run until 20th September 1988 and be made on the Grade III salary scale (£5,968 — £7,040).

Applications, in writing, including a detailed Curriculum Vitae and naming two referees, should be sent to Ms S N Dilks, Technical Manager, Leicester Biocentre, University of Leicester, Leicester LE1 7RH. (2291)A

ASSISTANTSHIPS

Volkswagenwerk Postdoctoral Research Assistantship

Applications are invited for a Postdoctoral Research Assistantship to work on a joint research project with the centre for mathematical biology (Mathematical Institute, University of Oxford), the Max-Planck-Institute (Dortmund) and the theoretical physics division (Harwell Laboratory) on numerical problems associated with nonlinear wave phenomena and spatial pattern formation in chemical and biochemical reaction diffusion systems. The successful applicant will be based in Oxford but will be expected to spend some time in the Max-Planck-Institute in Dortmund.

Applicants should have appropriate qualifications in applied mathematics, physics or engineering with experience in the numerical solution of partial differential equations. The appointment is for one year, renewable for a second year, from a mutually agreed starting date. Salary, dependent on qualifications and experience, within the range 9,000 to 11,500 pounds.

Applicants should send a curriculum vitae and the names of two referees, by 1st April 1987, to:

Administration Office of the Max-Planck-Institut Fuer Ernaehrungsphysiologie, Rheinlanddamm 201 4600 Dortmund 1, FRG. (3880)P

ANALYTICAL MICROSCOPIST

A vacancy exists in the microscopy section of the Analytical Group for a qualified Chemist or Physicist, preferably with a polymer bias, having a strong interest in the fields of light and electron microscopy.

The successful applicant will use these techniques to solve practical problems for industry as well as carrying out fundamental studi in areas of research such as pol mer composites.

Prospects for advancement a good for the right candidate.

Applicants should write, enclo ing a CV to:– The Director (r MJL), Malaysian Rubber Produ ers' Research Association, Tı Abdul Razak Laboratory, Bric endonbury, Hertford SG13 8NL (2293)A

STUDENTSHIPS

MRC BRAIN METABOLISM UNIT

University Department of Pharmacology,
1 George Square, Edinburgh EH8 9JZ

RESEARCH STUDENTSHIPS

Applications are invited from final year students expecting to graduate with a first or upper second class honours degree in appropriate branches of science, medicine or veterinary medicine. The successful applicants will work on one of the following topics: molecular and cellular mechanisms in the regulation of pituitary hormone secretion; steroid influences on gene expression; control of tachykinin gene transcription; role of serotonin in corticotrophin secretion; neuropharmacology of cerebral blood flow and metabolism; immunological studies of Alzheimer's Type Dementia and Down's syndrome. The applicants will be registered as research students in the University of Edinburgh.

Applications including a full curriculum vitae together with the names of two professional referees should be addressed to the Administrator, Mrs Anne Milligan, MRC Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ no later than April 6, 1987 (2176)F

UNIVERSITY OF EDINBURGH

DEPARTMENT OF BACTERIOLOGY

POSTGRADUATE STUDENTSHIP

Applications are invited for a three-year research scholarship, available from October 1987, to work on the association of *Pseudomonas aeruginosa* and pulmonary infection in patients with cystic fibrosis. The project will involve the use of various bacteriological and immunological techniques to investigate bacterial and host factors involved in pulmona colonisation with an aim to pr vide a raional basis for early dia nosis, antibiotic usage and vacci development. The successful ca didate will have (or attain th year) a 1st or 2nd class honou degree in microbiology, immun logy or related subject. Furth particulars can be obtained fro Dr John Govan, Department Bacteriology, Medical Schoo Teviot Place, Edinburgh EH 9AG (telephone 031 667 1011 ex 2211) to whom applications shoul be sent with a c.v. and the nam of two academic referees. (2284)F

A.F.R.C. UNIT OF NITROGEN FIXATION

RESEARCH STUDENTSHIPS

Two graduate studentships will become available in October 1987 to carry out research leading to a D. Phil. degree, at the Unit of Nitrogen Fixation. This is a multidisciplinary establishment carrying out fundamental research into nitrogen fixation, and is situated at the University of Sussex.

One studentship is to support work on genetic regulation of the recently discovered V-nitrogenase system in *Azotobacter chroococcum*. The project will entail the use of a variety of techniques in molecular genetics including recombinant DNA work, gene fusions, mutational analysis, and DNA sequencing.

The second student will be i volved in synthetic and mecha istic chemistry relevant to th nitrogen fixation problem, with particular emphasis on transitio metal hydrido-complexes. Th work will entail the use of a varie of techniques including the man pulation of air-sensitive co pounds and methods for studyi rapid reactions.

The successful applicants shoul hold, or expect to gain by Octob 1st 1987, a First or Second Cla Honours Degree in an appropria subject.

Please send curriculum vita with the names of two referees The Secretary, AFRC Unit Nitrogen Fixation, University Sussex, Brighton BN1 9RQ. Clo ing date 26 March 1987. (2267)F

STUDENTSHIPS

ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND

ANATOMICAL SOCIETY GRADUATE STUDENTSHIPS

Applications are invited for Studentships tenable in the Anatomical Sciences for a period up to three years. Nominees would be expected to work for a higher degree by research. Candidates must be graduates of British or Irish Universities and must have arranged sponsorship with a Department of Anatomical Sciences in the UK or Ireland. The stipend will be commensurate with basic Research Council rates.

ANATOMICAL SOCIETY SENIOR VISITING FELLOWSHIP

Applications from Overseas Scientists are invited for one Fellowship tenable for a period of less than a year in a Department of Anatomical Sciences in the UK or Ireland. Applicants should be of postdoctoral or comparable status and must have arranged sponsorship in the Department in which they intend to work. Some assistance towards travel and subsistence will be provided.

Further particulars of the Appointments should be obtained from the Honorary Secretary, Professor J A Gosling, Department of Anatomy, Stopford Building, University of Manchester, Oxford Road, Manchester, M13 9PT, to whom applications should be sent by July 15th 1987. Applications for studentships must be accompanied by sealed letters of support from two referees. (2122)F

FELLOWSHIPS

INTERNATIONAL CANCER RESEARCH FELLOWSHIP

The Hayashibara Mutual Aid Fund, a non-profit making organization within the Hayashibara Group announces a fellowship program. Two awards will be made annually. Fellowship will be taken up at the newly opened Fujisaki Cell Center which is devoted for the basic and applied research related to the problems in human cancer. The fellowship is normally made for one year and is renewable for up to five years. The fellowship will be expected to commence within six months of the announcement of the awards. The awards are for high quality research work in one of three categories described below.

1. Fundamental Leukemia-Lymphoma Research
2. Cytokine-Lymphokine Research
2. Hematopoietic Cell Cultures

Qualified person who has a PhD, an MD or equivalent qualifications will be awarded on merit to suitably qualified research proposal and experiences. Additional information and application forms will be obtained from: **Jun Minowada, M.D., Director, Fujisaki Cell Center, 675-1, Fujisaki, Okayama 702, Japan.** (W3636)E

Please mention

nature

when replying to

these advertisements

RESEARCH FELLOWSHIP IN CARDIOVASCULAR DISEASES

Two-year position available 1 July 1987 under NIH Training Grant, focusing on mechanisms of and therapy for arterial thrombosis and relationship to rheology, vasoconstriction, and pathologic substrate in animals and humans. Candidates should be partially or fully trained clinical cardiologists; U.S. citizenship or immigrant visa required. Supplemental clinical cardiovascular training can be considered. **Send curriculum vitae, statement of research interests, and names, of three references to: James H. Chesebro, M.D., Mayo Clinic, Rochester, MN 55905.** *An Equal Opportunity/Affirmative Action Institution.*

(NW600)E

NATIONAL CANCER INSTITUTE

National Institutes of Health

CANCER PREVENTION FELLOWSHIP PROGRAM (CPFP)

The Division of Cancer Prevention and Control is accepting fellowship applications for a proposed August 31, 1987, entry into CPFP. Funding permitting, up to ten individuals may be accepted for three years of training. Requirments are: M.D., D.O., or an accredited doctoral degree in a discipline related to cancer prevention and research (medicine, biomedical or behavioral research, nutritional science) or equivalent; academic-professional excellence supported by official transcripts; four letters of reference; and U.S. citizenship or a resident alien eligible for citizenship within four years at the time of application. *Current salary ranges for an M.D./D.O. or a Ph.D., respectively, are $26,000 — $35,000 or $18,000 — $31,000 per annum.* Send a postcard with your name and address to **Ms. Nancy E. Garner, NIH/NCI/DCPC/CCAB, Blair Building, 9000 Rockville Pike, Room 4A01, Bethesda, MD 20892–4200**, or call (301) 427-8788 for application and information packet.

DEADLINE FOR RECEIPT OF APPLICATIONS IS MARCH 16, 1987.

An Equal Opportunity Employer.

(NW507)E

POSTDOCTORAL FELLOWSHIP

A vacancy exists for a postdoctoral fellow interested in studying the regulation of immunity to tumour transplantation antigens and tumor histocompatibility antigens. Applicants should have a basic training in immunology. Starting salary will be $18,500 plus liberal fringe benefits. Subsidized Institute housing is available.

The Trudeau Institute is a private, not-for-profit, basic medical research institution located on the shore of Saranac Lake and near Lake Placid, NY.

Send curriculum vitae and names of three references to: Dr Robert J North, Director, Trudeau Institute, Inc., P.O. Box 59, Saranac Lake, NY 12983. (NW578)E

EMBO

EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

SHORT TERM FELLOWSHIPS

in molecular biology

The European Molecular Biology Organization awards, to scientists working in Europe and Israel in the field of molecular biology and allied disciplines, short term fellowships of one week up to three months duration. The fellowships are to support collaborative research between laboratories in different countries and provide a travel grant and subsistence allowance. Applications may be made at any time and are decided upon soon after the receipt of application.

Applications for exchanges between laboratories within any one country cannot be considered. Fellowships involving transatlantic travel are awarded only in extremely exceptional circumstances. Inquiries should be accompanied by a self-addressed adhesive label.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022. 40, F.R.G. (W3679)E

FELLOWSHIPS

THE FLINDERS UNIVERSITY OF SOUTH AUSTRALIA

School of Biological Sciences

RESEARCH FELLOWSHIP IN BIOLOGY

Applications are invited from suitably qualified persons for a Research Fellowship tenable within any of the fields represented in the School of Biological Sciences of the University. The Research Fellowship, which is available from 30 June 1987, is tenable initially for one year, but application may be made for an extension of up to a further twelve months. Applicants should have completed a PhD Degree prior to taking up the appointment, or have had equivalent research experience.

The appointment will be made according to qualifications and experience within the salary range $A24,013–$A27,507. Persons with a PhD will normally be appointed on the third step, $A25,414. A contribution will be made towards the travel costs of the Fellow and his/her dependents in taking up the Fellowship.

The School of Biological Sciences is an integrated school, without subdivisions into separate departments. Research conducted in the School includes the following fields of study: Behavioural Biology (animal models of depression and dementia, hormonal influences on dopamine sensitivity); Biochemistry (regulation of growth, carcinogenesis, the structure of the human genome, its repetitive sequences and their inter-relation, chromatin sub-structure, plant hormones, phytochrome action, differentiation in slime moulds); Biophysics (proton and electron flow in photosynthesis, ion channels in plant membranes, electrophysiology of fast movement in plants, vocal communication in animals and birds); Developmental Biology (germ plasm in Anura, reproductive embryology); Genetics (population genetics, radiation and chemical mutagenesis, genetics of Drosophila compound chromosomes, Drosophila development); Immunology (mechanisms of self-tolerance, and of autoimmune disease); Microbiology (mechanisms of the anti-tumour action of interferon, herpes viral antisera, molecular genetics of Neurospora, novel uses of fungi, Pseudomonas genetics); Animal Physiology (comparative cardio-respiratory physiology, physiological scaling, ecophysiology of marsupials); Population Biology (ecological interactions of closely related species, parapatric boundaries, plant-ant interactions, behavioural ecology of host selection by insects); population of smut fungi, Vertebrate palaentology (late Pleistocene marsupials, environments and climatic change); Rangelands Ecology (sustained co-existence of marsupial herbivores and domestic stock in arid lands); Plant Systematics and Plant Breeding Systems.

Further details concerning the staff of the School and their fields of interest are obtainable from the Dean, The School of Biological Sciences, The Flinders University of South Australia, Bedford Park, S.A., 5042, Australia.

Applications, including full personal details, details of academic record and publications, a brief outline of research interests and names of at least two referees, should be lodged, in duplicate, with the Registrar by 21 April, 1987. Each applicant should ask his or her referees to forward their reports directly to the Registrar before the closing date. (W3873)E

UNIVERSITY OF KENT AT CANTERBURY

Postdoctoral Fellow in Biological Chemistry

Applications are invited for postdoctoral research assistantships in Italy and England on the synthesis of organic intermediates catalysed by modified enzymes soluble in organic solvents.

The work is part of a collaborative project between Kent and the University of Genoa funded by the EEC.

The appointments are open to EEC nationals and are for one year renewable for a further year. Salary: Italy — L22,000,000 in the first year and L23,100,000 in the second; England — in the range of £8,020—£12,780 per annum (under review). The starting dates are negotiable within 1987.

Contact Dr A. Williams, Chemical Laboratory, Univerity of Kent CT2 7NH for further details.

Apply by letter with full curriculum vitae and the names and addresses of two referees to the Senior Assistant Registrar, Faculty of Natural Sciences, The Registry, The University of Kent, Canterbury, Kent CT2 7NH. Please quote reference A87/33. (2260)P

UNIVERSITY OF SUSSEX

School of Biological Sciences

Research Fellowships

Applications are invited for four Post-Doctoral Research Fellowships in the School of Biological Sciences, working on the following projects.

1. GENE EXPRESSION IN PARASITIC PROTOZOA — Dr. T.J.C. Beebee

Applications are invited for a post-doctoral position to run for at least two years, starting as soon as possible (subject to mutual arrangement). The project concerns the use of monoclonal antibodies to study gene expression in parasitic protozoa (trypanosomes) and is cooperatively funded by the MRC and Wellcome Research laboratories. Previous experience with either trypanosomes or monoclonal antibodies would be advantageous but is not essential. Salary within the range of £8,020 to £9,495 on the 1A scale depending on age and experience.

2. CELL CYCLE CONTROL IN XENOPUS — Dr. C.C. Ford

This post is funded by the SERC for three years to work on the regulation of early embryonic cell cycles in Xenopus. The project will involve analysis of protein synthesis and post-translational modifications during interphase and during interphase-mitosis-interphase transitions. Use will be made of microinjection techniques as well as a cell-free replication system and MPF extracts. Experience of microinjection, protein synthesis, protein modification or replication assays would be preferred but not essential. Salary within the range of £8,020 to £9,495 on the 1A scale and the post will be available from 1 March 1987.

3. PROTEIN ENGINEERING OF β-LACTAMASES AND PENICILLIN-BINDING PROTEINS — Dr. B.G. Spratt

This post is funded by the EEC and the successful applicant will be based at Sussex University but will work in close collaboration with enzymology and X-ray crystallography groups at the University of Liège, Belgium (Prof. J-M Ghuysen, Drs. J-M Frère and O. Dideberg). Applicants should preferably have a strong background in molecular genetics or protein chemistry. The position is funded for two years and 9 months from 1 April 1987 in the salary range £8,020 – £12,780 on the salary scale 1A.

4. CYTOSKELETAL PROTEINS IN YEAST —Dr. F. Watts

This post is funded by the SERC to study the structure and molecular genetics of the yeast cytoskeleton. The project will involve the use of recombinant DNA techniques and immunological methods. Applicants should have a background in Biochemistry or a related subject. Experience in recombinant DNA methods would be preferred but is not essential. The salary scale is on the range £8,020 – £9,495 on the 1A scale and the post will be available from 1 March 1987.

Applicants should send a curriculum vitae (including a list of publications) with the names and addresses of two referees to Mrs. N. Ford, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, Sussex. Telephone (0273) 606755. Additional information may be obtained from Dr. Beebee (Ext. 2690), Dr. Ford (Ext. 2850), Dr. Spratt (0273 678309), Dr. Watts (Ext. 2681). (2296)E

DEPARTMENTS OF CELLULAR AND DEVELOPMENTAL BIOLOGY ORGANISMIC AND EVOLUTIONARY BIOLOGY HARVARD UNIVERSITY

POSTDOCTORAL FELLOWSHIPS

A limited number of postdoctoral research positions become available from time to time. Some appointments are funded through research grants awarded to faculty members and are ordinarily for one year (sometimes renewable), other appointments are possible through postdoctoral fellowships. For a list of faculty members who have postdoctoral positions available, write to the respective departmental office (do not send applications): **Harvard University, 16 Divinity Avenue, Cambridge, MA 02138.**

Harvard University is an Equal Opportunity Affirmative Action Employer. (NW593)E

FELLOWSHIPS

POST DOCTORAL RESEARCH FELLOWSHIPS IN MOLECULAR BIOLOGY AND GENETICS

Several post doctoral fellowships are available to individuals with doctoral training in molecular biology. Studies include molecular/cell biology and molecular genetics of lysosomal glycoproteins; characterization of oncogenes and their gene products; and studies of protein folding and translocation. Appointments are made for 2 years.

Please send résumés to: **Division of Medical Genetics, CHILDRENS HOSPITAL OF LOS ANGELES, 4650 Sunset Blvd., Los Angeles, CA 90027, Attn: John A. Barranger, Ph.D., M.D.**

(NW603)E

Sandoz Institute for Medical Research
Gower Place, London WC1

Post-Doctoral Fellowship in Biochemistry

Applications are invited for a post-doctoral fellowship tenable for up to 2 years. The successful candidate will join a group working on the isolation, purification and characterisation of the bradykinin receptor from CNS and other relevant tissues. The work will be under the supervision of Dr. C.R Snell and will involve affinity purification using immobilised ligands or antibodies, partial sequencing and the use of cDNA probes and molecular cloning to obtain the total sequence of the receptor protein. Previous experience of receptor purification, microsequencing or DNA technology would be an advantage, although is not essential.

The post offers the opportunity to work in a multi-disciplinary environment concentrating on a range of basic neurobiological problems, as well as projects aimed at the development of potential new analgesic drugs.

Salary will be in the range £11,000-£15,000 p.a. Applications with the names of two referees should be sent to Mrs. M. C. Stuart, Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN by 5th April 1987.

SANDOZ

(2271)E

Lucille P. Markey Visiting Fellowships Program

Nominations are invited for postdoctoral fellowships under the above program. Up to four awards for a period of two years each will be made to outstanding candidates wishing to further their careers in biomedical science at a research institution in the U.S.A. Each awardee will receive a $25,000 stipend (plus $750 travel allowance) in the initial year, with a $3,000 increase in stipend for the second year. Candidates will be expected to specify the institutions at which they hope to work, the name of the preceptor under whose direction the work will proceed and obtain a commitment from the institution and preceptor that appropriate facilities for the research will be provided. The U.S. institution must have the appropriate tax exemption. Under the terms of the Lucille P. Markey Charitable Trust support is given for the study of fundamental biological processes rather than of specific disease entities and most awards will support work in fields such as molecular biology, developmental biology, neurobiology, immunobiology, immunology, genetics, virology and related areas of basic science.

Candidates *must* be nominated by a university or research institution in the U.K. and individual applications cannot be considered.

Further information and application forms can be obtained from:

Dr G. Stark
Imperial Cancer Research Fund
P.O. Box 123
Lincoln's Inn Fields
London WC2A 3PX
Closing date for completed applications: 1st June 1987.

(2252)E

ROCKEFELLER FOUNDATION BIOTECHNOLOGY CAREER FELLOWSHIPS

The Rockefeller Foundation announces a program of career development fellowships designed to enable scientists from developing countries, trained at outstanding centers for advanced research in biotechnology, to continue to work at those or other institutions for three months each year, over a period of at least three years, conducting advanced research and keeping abreast of new developments in their fields. The program will focus upon the development and application of advances in molecular and cellular biology and immunology relevant to agriculture, health, and reproductive biology.

Funding will be shared between the Foundation and the host laboratory, with the Foundation providing travel and per diem support. It is hoped that the fellowships will encourage the establishment of ongoing working relationships between outstanding younger scientists working at third world institutions, and research teams at advanced laboratories.

Applicants to this program should have at least Ph.D.- or M.D.-level training, a proven record of scientific productivity, and a permanent position at a research or teaching institution in their home country. A written project proposal must be developed and submitted jointly by the candidate and the laboratory sponsor.

Information about application procedures can be obtained by writing to **Biotechnology Career Fellowships, Fellowship Office, Rockefeller Foundation, 1133 Avenue of the Americas, New York, New York 10036 USA.**

(NW566)E

GRANTS & SCHOLARSHIPS

NATIONAL UNIVERSITY OF SINGAPORE

RESEARCH SCHOLARSHIPS

Research Scholarships are available to outstanding university graduates registered for, or admissable to, master's and doctor's degree programmes by research at the National University of Singapore.

The Scholarships are tenable for one year in the first instance and, subject to satisfactory progress, renewable annually for another two years.

The value of each Scholarship ranges from **S$800** to **S$1100** per month (£1.00 =S$3.25 approximately) and is not subject to income tax; the actual quantum is determined on the candidate's qualifications and experience. No travel or other allowance is provided.

Research Scholars may be asked to assist in the work of the Departments to which they are attached for not more than three hours a week. They will be remunerated for the work done.

The University will help Research Scholars find off-campus accommodation.

Application forms may be obtained from:

NUS Overseas Office
5 Chesham Street
London SW1
United Kingdom

The Registrar
NATIONAL UNIVERSITY OF SINGAPORE
10 Kent Ridge Crescent
Singapore 0511
Republic of Singapore

In your request for application forms, please state —
(a) the field of research you intend to undertake; and
(b) your highest educational qualification.

Applications must be received by the Registrar, National University of Singapore by **30 May 1987.**

(W3855)H

AUSTRALIAN BICENTENNIAL 1788-1988

GRANTS FOR STUDY/ RESEARCH IN AUSTRALIA

The Science, Technology, Industry and Medicine (STIM) Subcommittee of the Britain-Australia Bicentennial Committee is offering a limited number of awards for permanent UK residents of post-doctoral or equivalent status engaged in scientific, technological, engineering or medical research to make study visits of between 6 and 10 weeks duration to Australia in the Bicentennial Year (1988).

The object is to stimulate collaborative links between researchers and to provide an opportunity the particular strength of Australian research and/or the unique environmental features of the country. Grants will provide assistance with air fares, living costs and possibly expenses related to field work but are not likely to exceed £2,500 in total.

Applications can be considered from persons who will have attained PhD or equivalent status by the time of the visit, and who will not have passed their fortieth birthday by the time the visit is completed. Applications should be made on forms obtainable from the Chairman, STIM Subcommittee, (Ref. BdeV), c/o The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG (Tel. 01-839 5561, ext. 222) and submitted by the closing date of 30 September

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AWARDS

THE CIBA-GEIGY PRIZE FOR RESEARCH IN ANIMAL HEALTH

At the end of 1987, Ciba-Geigy Animal Health will consider submissions for the award of 50,000 Swiss Francs to a scientific worker whose research might be applied to the improvement of animal health through the prevention or treatment of disease.

A submission may be made by the originator of the work or a recommendation may be made by a third party.

The work must be original to the nominee and, while it need not have been carried out during the current year, it should be novel either in concept or development.

The closing date for entries will be 31 May 1987.

Further details and copies of the rules governing the award may be obtained from:

Dr. Patrick Barden FRCVS
or Dr. Rolf F. Steiger
CIBA-GEIGY Limited
R-1001.B.P.87
CH-4002 Basel
Switzerland

(W3443)N
13088

BIOTECHNOLOGISTS!

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Felicity Parker, Macmillan Journals,
4 Little Essex St.,
London WC2R 3LF
or
Paul Siman, Bio/Technology,
65 Bleecker St., New York, NY 10012.

CSH

COLD SPRING HARBOR LABORATORY CONFERENCE
ON
YEAST CELL BIOLOGY
August 11-August 16

Organized by

Amar Klar, *Cold Spring Harbor Laboratory*
Paul Nurse, *Imperial Cancer Research Fund*
Randy Schekman, *University of California, Berkeley*

A specialized international Yeast meeting emphasizing areas of cell cycle controls, developmental choices, cell- cell recognition, cytoskeleton and cell structure, protein targeting and modification, elements of chromosome structure and function, and other relevant areas will be held from August 11-16, 1987. Open call for abstracts. The abstract deadline is JUNE 2. For further information, contact:

Meetings Coordinator
Cold Spring Harbor Laboratory
P.O. Box 100
Cold Spring Harbor, NY 11724
(516) 367-8346

(NW565)C

EUROPEAN FEDERATION OF CHEMICAL SOCIETIES

EUROANALYSIS VI

TRIENNIAL EUROPEAN CONFERENCE
ON ANALYTICAL CHEMISTRY

ORGANIZED BY FRENCH CHEMICAL AND METALLURGICAL SOCIETIES

1987, SEPTEMBER 7-11 – PARIS (FRANCE)

Invited lectures will be given on Electron tunnelling microscopy and spectroscopy, the use of lasers for ultrasensitive analysis, structure and operation of ion selective electrodes, F.I.A., ion probe analysis, the use of Fourier transform analysis, Surface analysis, Problems in pollution, etc...

More than 400 contributed papers cover : Chromatography, Electrochemistry, all Spectroscopies, Pharmaceutical and biological analysis, etc... and their applications.

An exhibition of instruments, books,reagents, will take place.

The Official Language is english French to english simultaneous translation is contemplated.

The Site is the «La Villette international Conference Center of the Cité des Sciences et de l'Industrie».

Send your preregistration and ask for further details (exhibition space, accomodation etc...) now to

G A M S **88, Boulevard Malesherbes, 75008 PARIS (France)**

(W3862)C

The Third Princeton-Liposome Conference*

THERAPEUTIC APPLICATIONS OF LIPOSOMES

MAY 11-13, 1987 • PRINCETON, NEW JERSEY • SCANTICON CONFERENCE CENTER

CONFERENCE CHAIRMEN:

Dr. Sol M. Gruner (Princeton University) Dr. Marc J. Ostro (The Liposome Co., Inc.) Dr. Gerald Weissmann (N.Y.U. Medical Center)

SYMPOSIA AND SPEAKERS

SYMPOSIUM I: INFECTIOUS DISEASE • Chairman: Dr. George Mackaness (Squibb Institute for Medical Research)
Dr. Andrew Whelton (Johns Hopkins University)
Dr. Gerald P. Bodey (M.D. Anderson Hospital & Tumor Institute)
Dr. Richard S. Ginsberg (The Liposome Co., Inc.)
Dr. Gabriel Lopez-Berestein (M.D. Anderson Hospital & Tumor Institute)

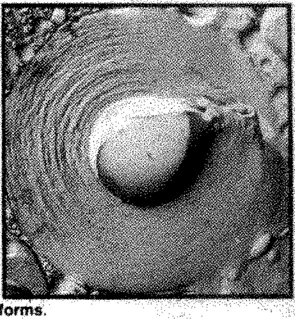
SYMPOSIUM II: INFLAMMATION • Chairman: Dr. Gerald Weissmann (N.Y.U. Medical Center)
Dr. Bengt Samuelsson (Karolinska Institute, Sweden)
Dr. Kurt Isselbacher (Harvard Medical School)
Dr. John Dingle (Strangeways Research Laboratories, U.K.)
Dr. Alan L. Weiner (The Liposome Co., Inc.)

SYMPOSIUM III: CANCER I • Chairman: Dr. Dr. Alan C. Sartorelli (Yale University School of Medicine)
Dr. Joseph Bertino (Yale University School of Medicine)
Dr. Victor Ling (Ontario Cancer Institute, Canada)
Dr. Pieter Cullis (University of British Columbia)
Dr. Roman Perez-Soler (M.D. Anderson Hospital & Tumor Institute)

SYMPOSIUM IV: CANCER II • Chairman: Dr. Rudolph L. Juliano (University of North Carolina, Chapel Hill)
Dr. Emil Frei (Dana Farber Cancer Institute)
Dr. Zanvil Cohn (Rockefeller University)
Dr. Peter Dukor (Ciba-Geigy, Ltd., Switzerland)
Dr. John Weinstein (National Institute of Health)

SYMPOSIUM V: VACCINES • Chairman: Dr. Keith J. Dorrington (Connaught Laboratories, Canada)
Dr. Richard Lerner (Scripps Clinic & Research Foundation)
Dr. Louis Chedid (Univ. of So. Florida College of Medicine)
Dr. Carl R. Alving (Walter Reed Army Institute of Research)
Dr. Howard Six (Baylor College of Medicine)

Senator Lowell Weicker from Connecticut will be the Special Guest Speaker on Monday, May 11 • 8 p.m. at the banquet.



★CO-SPONSORED BY THE BIOLOGY DEPARTMENT OF PRINCETON UNIVERSITY AND THE LIPOSOME COMPANY, INC.

Posters: Scientists interested in presenting **POSTER PAPERS** should submit a brief (250 words or less) abstract with their registration forms.

Payment: Registration fee must accept this registration form in order for a place to be reserved in your name.

(NW572)C

REGISTRATION	NON PROFIT	ALL OTHERS
3 Day Conference (includes double room, board, banquets, concert and conference fees)	$450	$850
3 Day Conference (includes single room, board, banquets, concert and conference fees)	$600	$1,000
Daily Conference (conference fees, luncheons for 3 days, concert and banquet)	$250	$600

Name ______________________ Title ______________________
Dept. ______________________ Organization ______________________
Street ______________________ City ______________________
State ______________________ Country ______________________
Zip ______________________ Telephone ______________________

Return to: M. Babice, Conference Coordinator, The Third Princeton-Liposome Conference, P.O. Box 9301, Trenton, N.J. 08650-1301

☐ Please check this box if you wish to purchase the book entitled "Liposomes, Biophysics to Therapeutics" being offered at a conference price of $35 (reg. price - $70)

FIRST ANNOUNCEMENT, CALL FOR POSTERS AND REGISTRATIONS

THIRD INTERNATIONAL CONFERENCE ON PROGRESS IN CANCER RESEARCH

SAN REMO, ITALY MAY 4–6, 1987

Organized by:

National Institute for Cancer Research — Genoa, Italy
Italian League Against Cancer

Scientific and Organizing Committee:

Renato Dulbecco, U.S.A. (Chairman)	Paul Marks, U.S.A.
Nikolai N. Blokhin, U.S.S.R.	Leonardo Santi, Italy
Vincent De Vita, U.S.A.	Luciano Zardi, Italy

General Secretary:

Giovanni Lotti, Italy

Conference topics will focus on:

Oncogenes — Growth Factors — Antioncogenes — Monoclonal Antibodies in Tumor Diagnosis and Therapy — Chemotherapy and Drug Resistance — Interferon Interleukin-2 and LAK Cells in Tumor Therapy — Reversion of the Malignant Phenotype — Cell-Cell Adhesion, Cell-Extracellular Matrix Adhesion and Metastasis — Tumor Stroma Generation

Invited speakers include:

Webster Cavenee, Canada; Luigi Chieco-Bianchi, Italy; Giuseppe Della Porta, Italy; Bernard Fisher, U.S.A.; Janice Gabrilove, U.S.A.; Judy Gasson, U.S.A.; Jordan Gutterman, U.S.A.; Henry Harris, England; Dan Longo, U.S.A.; Michael Lotze, U.S.A.; John Minna, U.S.A.; Rita Levi Montalcini Italy; Malcolm Moore, U.S.A.; Lorenzo Moretta, Italy; Pier Giorgio Natali, Italy; Kenneth Olden, U.S.A.; Ira Pastan, U.S.A.; Giorgio Prodi, Italy; Neal Rosen, U.S.A.; Jean Paul Thiery, France; Inder Verma, U.S.A.; Bengt Westermark, Sweden; Owen Witte, U.S.A.

Registration fee, including lunch, will be 350,000 Italian Liras (about 240 US Dollars). A limited number of fellowships will be available. Application form and abstract should be submitted before April 15, 1987 to:

M. Grazia Moro, Instituto Nazionale per la Ricerca sul Cancro, Viale Benedetto XV, 10, 16132 GENOVA, Italy. Tel. 39–10–352855 — Telex 216353 ISTEX 1

(W3882)C

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Information may be obtained from the Registrar, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT.

(2277)C

NATO Advanced Study Institute

FORMATION AND EVOLUTION OF LOW MASS STARS

21 September - 2 October 1987
Viana do Castelo, Portugal

Scientific Organizing Committee: A. K. Dupree, Director (U.S.A.), R. M. Bonnet (France), L Hartmann (U.S.A.), M.T.V.T. Lago (Portugal), J. Pringle (U.K.), H. Volk (F.R.G.)

The Institute will be devoted to one of the most challenging problems in contemporary astrophysics: formation and evolution of low mass stars (like the Sun) from both an observational and theoretical viewpoint. The course format, will consist of lectures, poster papers and discussions addressing current scientific techniques and results.

About 100 participants are contemplated and active researchers in the field are encouraged to attend.

To apply: request application form from Dr. A. K. Dupree, Harvard-Smithsonian Center for Astrophysics, MS-15, 60 Garden Street, Cambridge, MA 02138, U.S.A..

Partial financial support may be available.

Deadline for Application: **May 1**, 1987

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Further information and application forms from: **Dr. D.E. KIDD, Dept. of Physics and Applied Physics, University of Strathclyde, 107 Rottenrow, Glasgow G4 0NG, Scotland.**

(Closing date for applications 1st May 1987) (2158)C

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Further information contact:

Dr P. McCormack, MD, PhD.
Division of Life Sciences (CODE EB)
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Telephone 202 453–1543

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Applications are invited from medical and suitably qualified science graduates. Financial assistance towards fees is available on a competitive basis for overseas (non EEC) M.Phil and PhD students.

For further details please contact The Secretary, Department of Biochemistry, Institute of Psychiatry, De Crespigny Park, London SE5 8AF. Tel: (01) 703 5411 Ext 140. (2259)C

WORKSHOPS

WELLBORE SAMPLING WORKSHOP

May 27 – 28, 1987

(Houston, Texas, near Intercontinental Airport)

Workshop on Scientific wellbore sampling techniques to identify and prioritize the equipment needs to obtain scientific solid, fluid, and gas samples from drilled boreholes both in ocean basins and on deep continental drilling projects.

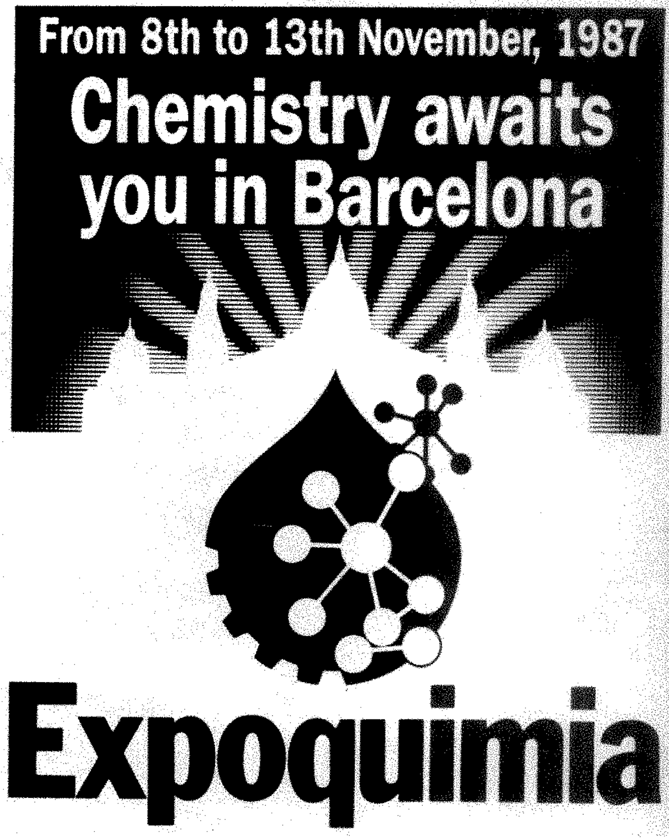
SPONSOR: Joint Oceanographic Institutions, Inc.; U.S. Science Advisory Committee; and the Department of Energy/Basic Energy Science. Contact for Information: R.K. Traeger — Sandia National Laboratories, Albuquerque, New Mexico (505) 844-2155; or B.W. Harding — Ocean Drilling Program, Texas A&M University, College Station, Texas (409) 845-2024.

The workshop will (1) summarize the current state of need for borehole samples to be used in geoscience research, (2) summarize the state-of-the-art in sampling technologies, and (3) use the interactions of geo-scientists and engineers to prioritize hardware needs for effective borehole sampling.

The workshop will include formal presentations, poster sessions, and a display of sampling hardware.

Applications are invited from potential workshop participants. Funds from a JOI-USSAC contract will be distributed to defray travel and subsistence expenses on the basis of applicant's eligibility and statements of interest and need.

Applications for funding must be received by May 15, 1987 — addressed to B.W. Harding, TAMU Research Park, 1000 Discovery Drive, College Station, TX 77840. (NW604)V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The first meeting devoted solely to the Na-Ca exchange system will be held at Stowe from July 12-16th. The meeting will cover: Partial Reactions of the Exchanger; Inhibitors and activators; Exchange fluxes in organelles; Stoichiometry, electrogenicity and sensitivity to voltage; Modelling; Molecular biology and the Role of the exchanger in normal and abnormal cell function.

Further details from Professor P. F. Baker, Department of Physiology, King's College London, Strand, London WC2R 2LS, U.K. (2298)M

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BIOTECHNOLOGY

NATURE'S 9TH INTERNATIONAL CONFERENCE & EXHIBITION • KENSINGTON TOWN HALL • LONDON • JULY 2 & 3 1987

CONFERENCE PROGRAMME

Thursday July 2
ANIMAL IMPROVEMENT

8.30 **Registration**
9.10 **Introduction,** John Maddox, *Nature*
9.15 **Transgenic techniques** R.M. Moore/D.J. Powell, *AFRC Institute of Animal Physiology & Genetics Research, Cambridge, England*
10.00 **Poultry improvement** G. Bulfield, *AFRC Institute of Animal Physiology & Genetics Research, Edinburgh, Scotland*
11.15 **Milk as a source of transgene products** J. Clark/P. Simons, *AFRC Institute of Animal Physiology & Genetics Research, Edinburgh, Scotland*
12.00 **Genetic enhancement of food and fibre value** G. Goldspink, *Royal Veterinary College, London, England*
12.45 **Lunch and exhibition viewing**

PLANT IMPROVEMENT

2.15 **Nitrogen fixation** J. Postgate, *Sussex University, England*
3.00 **Seed proteins** B. Miflin, *Ciba-Geigy, Basel, Switzerland*
3.45 **Tea and exhibition viewing**
4.15 **Monocotyledons** B. Hohn, *Friedrich Miescher Institute, Basel, Switzerland*
5.00 **Mitochondria and cytoplasmic sterility** C. Leaver, *Edinburgh University, Scotland*
5.45 **Cocktail reception**

Friday July 3
ANIMAL PROTECTION

9.10 **Introduction**
9.15 **Foot and mouth disease vaccines** F. Brown, *Wellcome Biotech, Pirbright, England*
10.00 **Rabies vaccines** J. Lécocq, *Transgène, Strasbourg, France*
10.45 **Coffee and exhibition viewing**

PLANT PROTECTION

11.15 **Baculoviruses** D. Bishop, *NERC Institute of Virology, Oxford, England*
12.00 **Frost protection** S. Lindow, *University of California, Berkeley, USA*
12.45 **Lunch and exhibition viewing**
2.15 **Viral resistance** D. Baulcombe, *AFRC Plant Breeding Institute, Cambridge, England*
3.00 **Herbicide resistance** G. Kishore, *Monsanto, St. Louis, USA*
3.45 **Bacterial and fungal resistance** R. Dixon, *Royal Holloway College, Surrey, England*
4.30 **Closing summary**

There will be ample opportunity for discussion within the programme as each formal presentation will be followed by a question period.

POSTER SESSIONS will be held each day. Anybody wishing to present a poster should send a title and a brief synopsis to Dr. Peter Newmark, Deputy Editor, **Nature,** 4 Little Essex Street, London WC2R 3LF, UK

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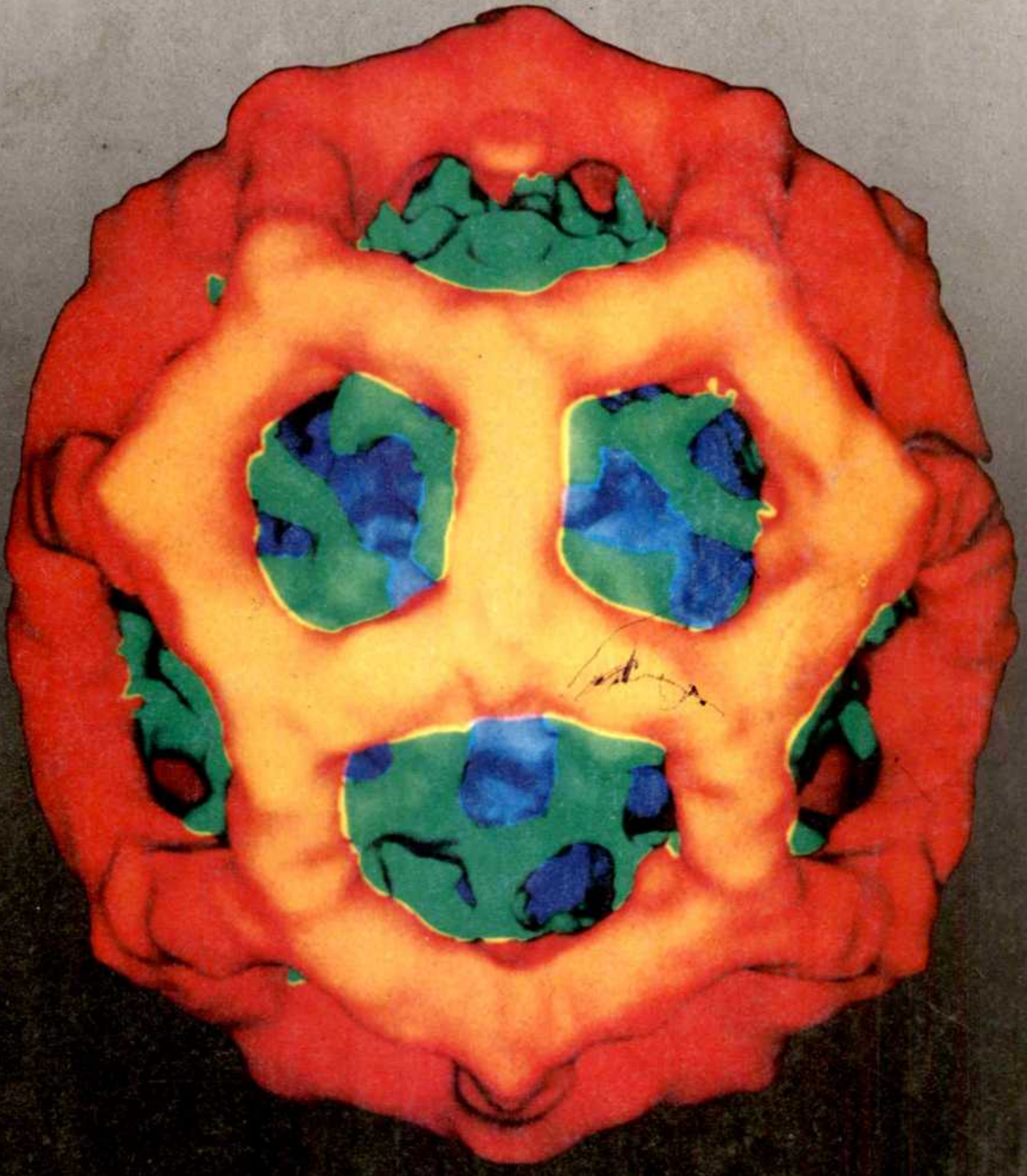

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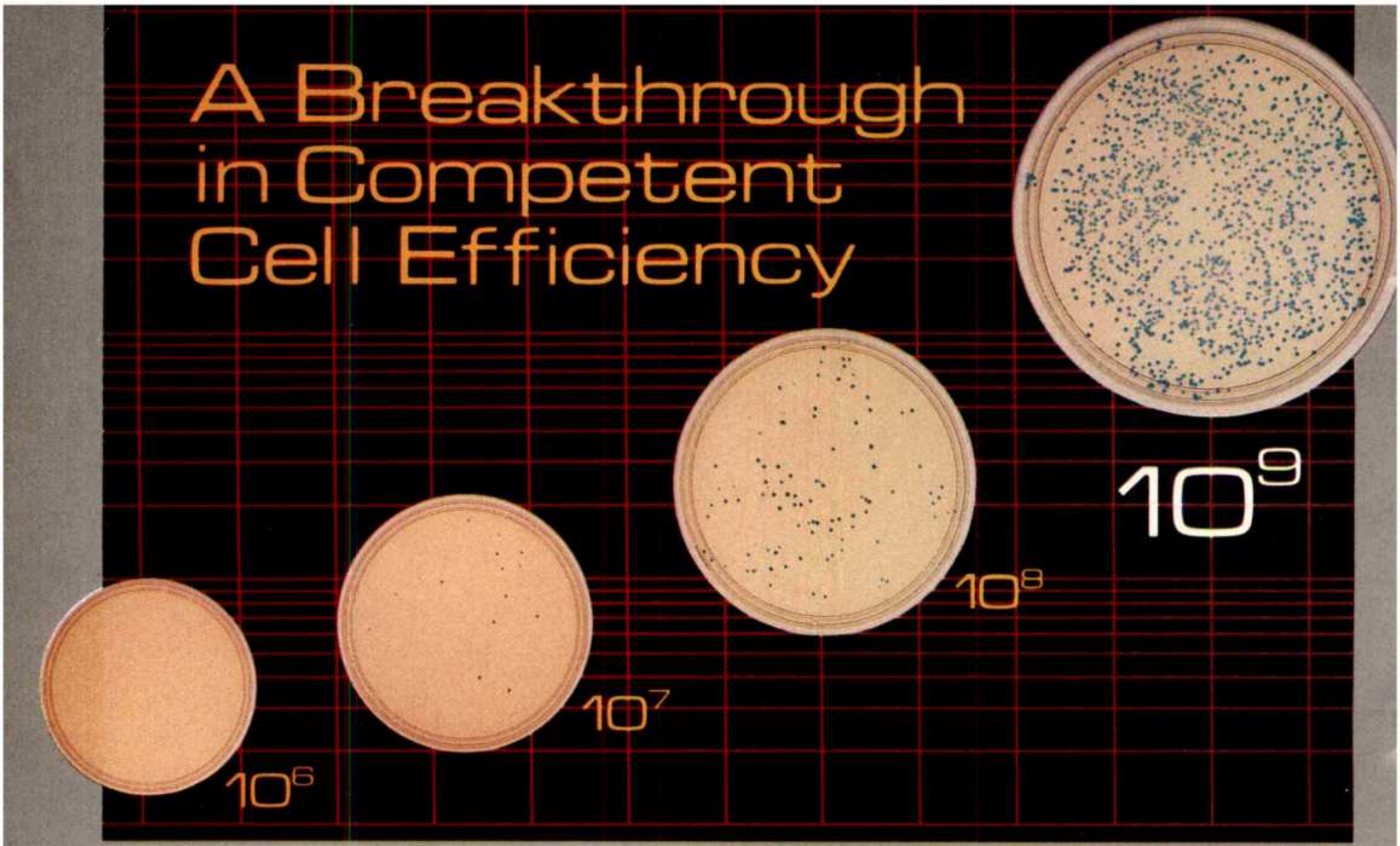

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XL1-Blue	YES	$\geq 3 \times 10^8$	YES[c]	endA1,hsdR17(r_k^-,m_k^+),supE44,thi-1,λ^-,recA1, gyrA96,relA1,Δ(lac),[F',proAB,$lacI^qZ\Delta M15$,Tn10(tet^R)].	1,2
AG1[d]	NO	$\geq 10^8$	NO	F^-,endA1,hsdR17(r_k^-,m_k^+),supE44,thi-1,λ^-,recA1,gyrA96,relA1.	1,2
NM522	YES	$\geq 10^8$	YES	Δ(lac-proAB),thi,hsdΔ5,supE,[F',proAB,$lacI^qZ\Delta M15$].	3
JM101	YES	$\geq 10^8$	YES	supE,thi,Δ(lac-proAB),[F',traD36,proAB,$lacI^qZ\Delta M15$].	4
JM109	YES	$\geq 10^8$	YES	recA1,endA1,gyrA96,thi,hsdR17,supE44,relA1, λ^-,Δ(lac-proAB),[F',traD36,proAB,$lacI^qZ\Delta M15$].	4

a. per μg pBR322 test plasmid
b. necessary for M13 infection
c. improved F' episome contains tet^R marker
d. high efficiency variant of DH1 (references 1,2)

REFERENCES

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2. Hanahan, D. 1983, J.Mol.Biol. 166:557-580.
3. Gough, J. and Murray, N. 1983, J.Mol.Biol. 166:1-19.
4. Yanisch-Perron, C., Vieira, J., Messing, J. 1985, Gene 33:103-119.
5. Fernandez, J., Short, J.M., Renshaw, M., Huse, W.D., and Sorge, J. 1987, Gene (submitted).

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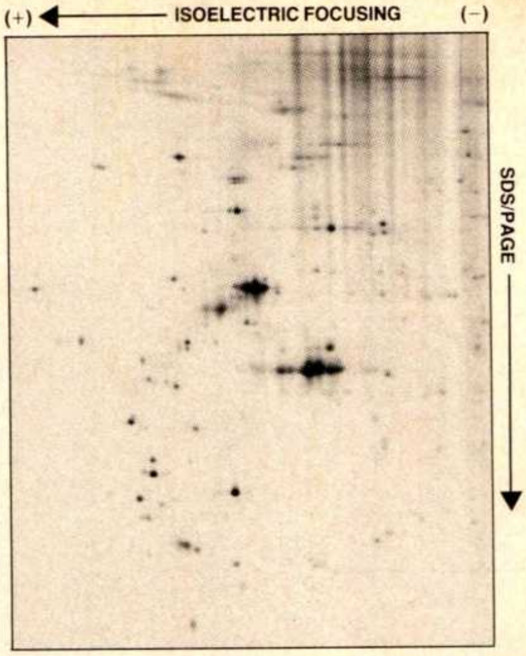

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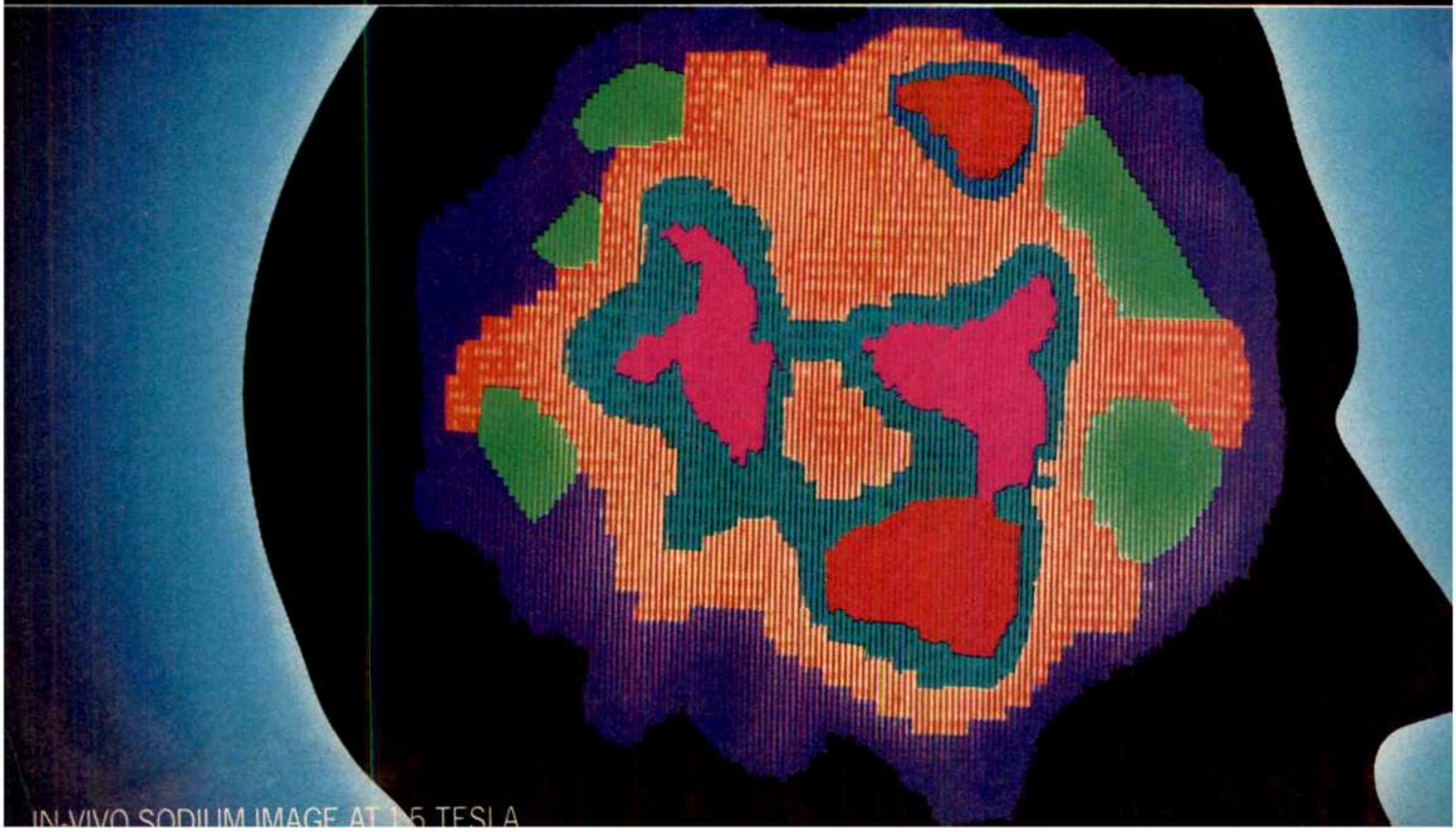

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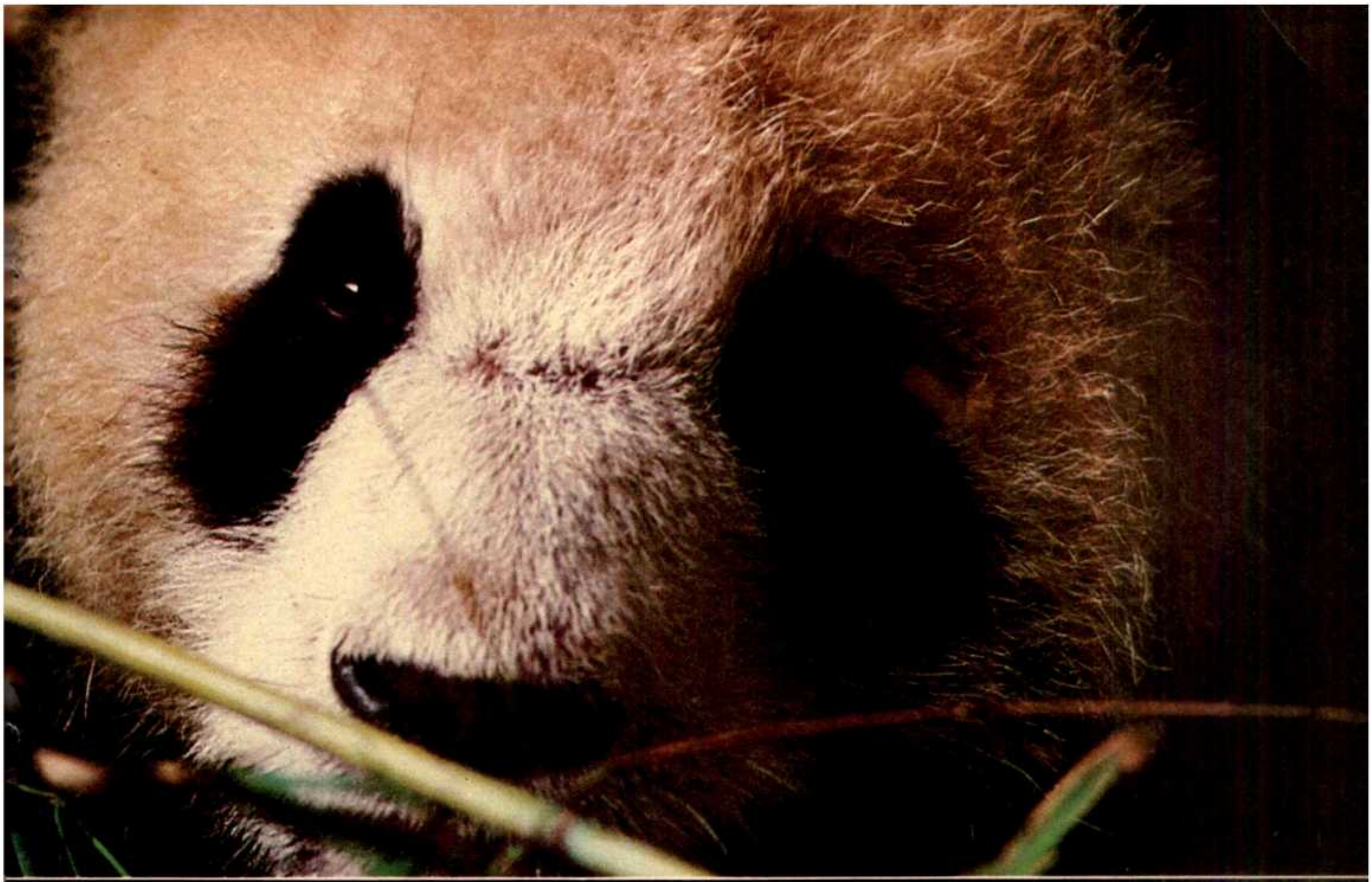

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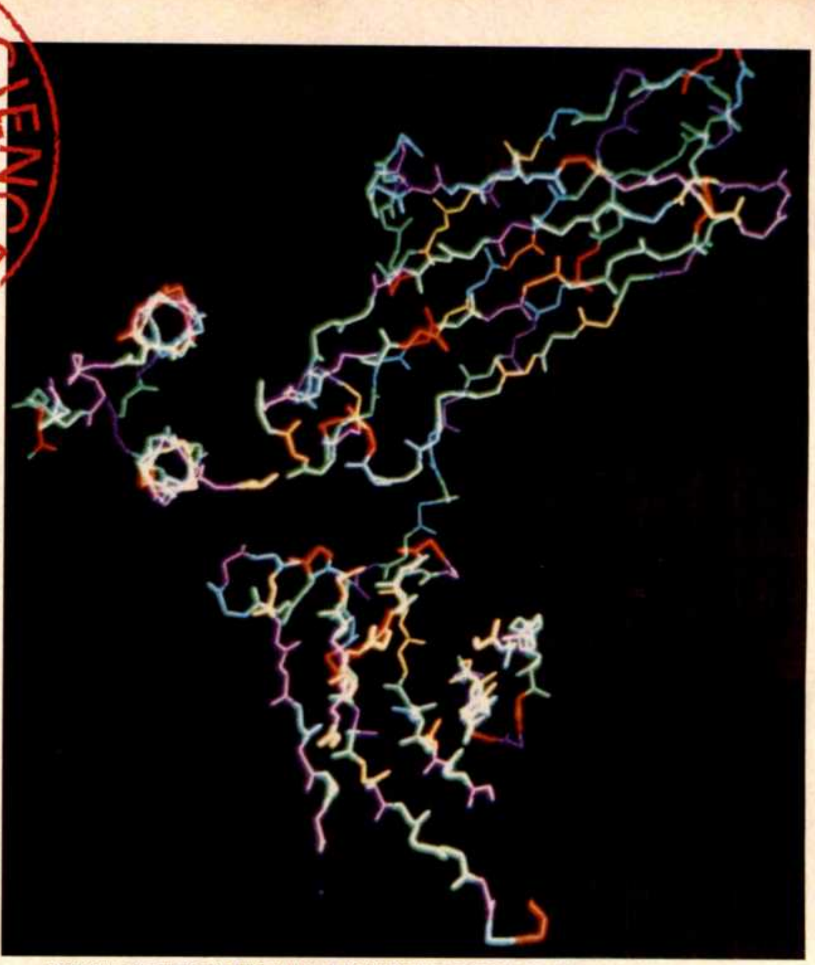

PROTEIN A, TO LEFT OF PICTURE, INTERACTING WITH THE Fc PORTION OF AN IgG MOLECULE. THE HINGE REGION IS CLEARLY VISIBLE. THREE DIMENSIONAL COMPUTER MODEL DERIVED USING FRODO SOFTWARE (T. ALWYN JONES) AND BASED ON X-RAY CRYSTALLOGRAPHY DATA (J. DEISENHOFER, *BIOCHEMISTRY 20*, 1981, 2361-2370) SUPPLIED BY THE PROTEIN DATA BANK.

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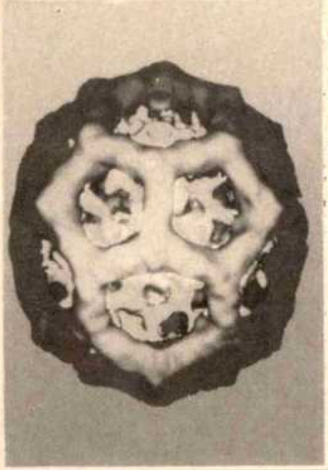


nature

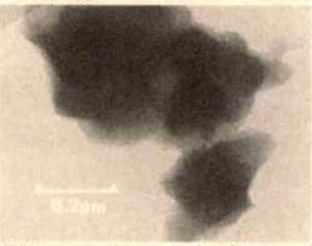
12 March 1987
Volume 326
Issue no. 6109

Three-dimensional map of a clathrin coat generated from electron micrographs. Colours highlight the outer polyhedral clathrin lattice (red), the shell of clathrin terminal domains (green) and an inner shell of accessory proteins (blue). (G.P.A. Vigers, R.A. Crowther and B.M.F. Pearse, *EMBO Journal* **5**, 2079 1986.)

THIS WEEK

■ Diamonds from an ancient star

. . .page 160



Tiny particles of diamond found in meteorites contain xenon and krypton of anomalous isotopic composition which suggests formation near a red-giant star preceeding a supernova explosion.

■ Supernova neutrinos

. . .pages 121 and 135

A calculation suggests that the supernova explosion in the Large Magellanic Cloud two weeks ago should produce detectable numbers of neutrinos in at least two operational experiments.

■ New hopanoids

. . .pages 126 and 179

A well-preserved microbial lipid precursor has been isolated from the organic fraction of a 50-million-year-old shale.

Huntington's disease

. . .page 195

Huntington's disease appears to be the first human disease of genetically documented homozygosity that displays complete phenotypic dominance.

■ Towards a schistosomiasis vaccine

. . .pages 131 and 149

Cloning the gene for an antigen, from the adult schistosome, that induces antiparasitic responses *in vitro* and partial protection in laboratory animals may be a first step towards a vaccine.

■ Epidemiology of AIDS

. . .page 137

Computer models point to the important factors in the spread of AIDS infection but suggest that it is too soon to assess the seriousness of the epidemic.

■ AIDS settlement in dispute

. . .page 115

A report from Washington suggests those in the United States and France may be nearing agreement on the patent dispute over AIDS blood-tests.

■ Cambrian life

. . .pages 127 and 181

Fossils from the Lower Cambrian of Greenland indicate a soft-bodied Burgess shale-like fauna. Similarity with the much earlier and geographically distant Burgess shale fossils shows the evolutionary stability of this fauna.

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[1] Transforming growth factor type beta isolated from human platelets (hTGFβ) or from porcine platelets (pTGFβ1) is sequentially (first 50 a.a.) and biologically identical. Both give an ED_{50} of 0.1-0.4 ng on NRK (49F) cells when assayed as per Assoian *et al* JBC *258*, 7155, 1983, and 0.8-1.0 ng/ml on AKR (2B) after Tucker *et al* CAN RES *43*, p1581, 1983.

[2] pTGFβ2 is a structurally distinct second form of TGFβ (sequence homology 70% in the first 50 a.a. to hTGFβ/pTGFβ1). When assayed as above, it gives the same ED_{50}s on NRK and AKR cells. However, some cell lines can distinguish between pTGFβ2 versus pTGFβ1/hTGFβ.

[3] Human platelet derived growth factor h(PDGF) is assayed on Balb/c-3T3 and NIH-3T3 cells for H^3-thymidine incorporation as per Raines and Ross, Meth. in Enz. *109*, p749.

[4] Porcine PDGF is structurally very different from hPDGF (i.e. differs in M.W., subunit composition, N-terminus), it's activity is determined as in 3 above.

[5] Both the acidic and basic forms of bovine fibroblast growth factor are isolated from brain and assayed for H^3-thymidine incorporation on NR6-3T3 cells after Gospodarowicz, *et al* JBC, *253*, p3736, 1978.

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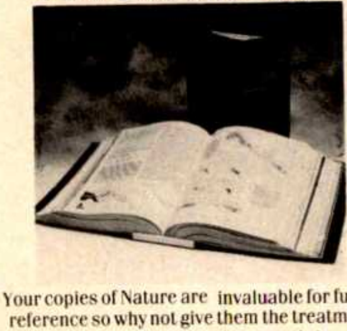

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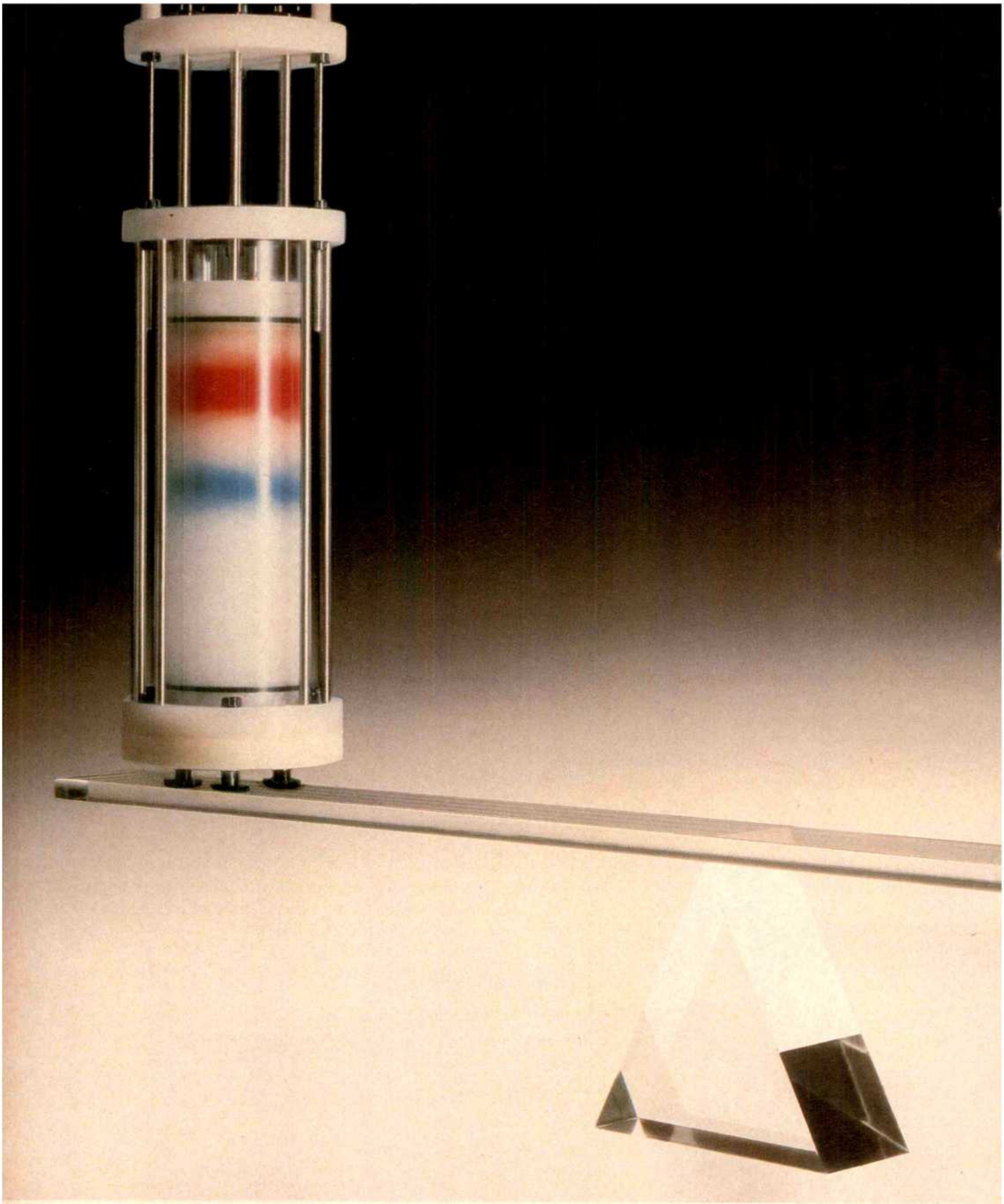

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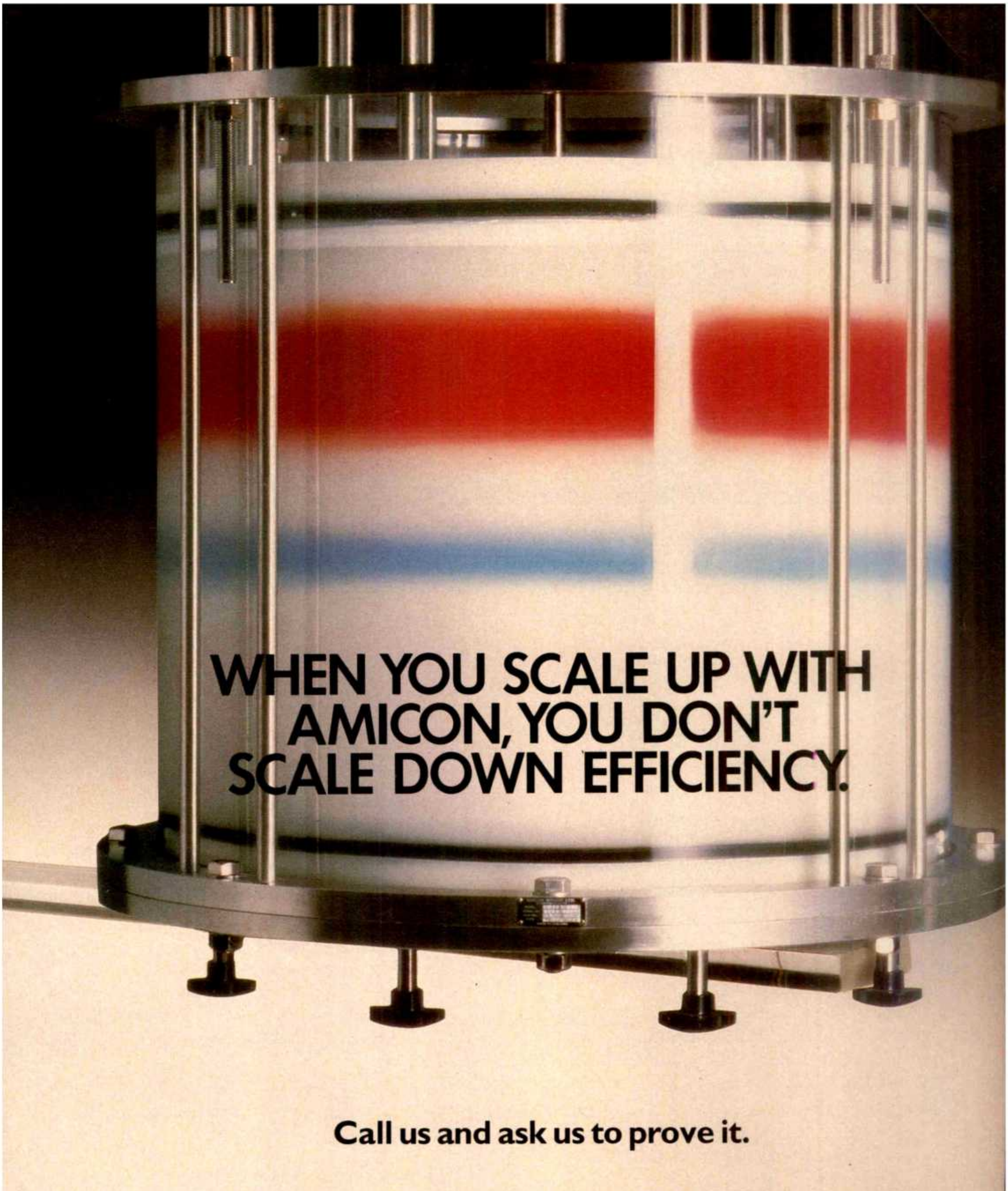
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CONFERENCE PROGRAMME

Thursday July 2
ANIMAL IMPROVEMENT

8.30 **Registration**
9.10 **Introduction,** John Maddox, *Nature*
9.15 **Transgenic techniques** R.M. Moore/D.J. Powell, *AFRC Institute of Animal Physiology & Genetics Research, Cambridge, England*
10.00 **Poultry improvement** G. Bulfield, *AFRC Institute of Animal Physiology & Genetics Research, Edinburgh, Scotland*
11.15 **Milk as a source of transgene products** J. Clark/P. Simons, *AFRC Institute of Animal Physiology & Genetics Research, Edinburgh, Scotland*
12.00 **Genetic enhancement of food and fibre value** G. Goldspink, *Royal Veterinary College, London, England*
12.45 **Lunch and exhibition viewing**

PLANT IMPROVEMENT

2.15 **Nitrogen fixation** J. Postgate, *Sussex University, England*
3.00 **Seed proteins** B. Miflin, *Ciba-Geigy, Basel, Switzerland*
3.45 **Tea and exhibition viewing**
4.15 **Monocotyledons** B. Hohn, *Friedrich Miescher Institute, Basel, Switzerland*
5.00 **Mitochondria and cytoplasmic sterility** C. Leaver, *Edinburgh University, Scotland*
5.45 **Cocktail reception**

Friday July 3
ANIMAL PROTECTION

9.10 **Introduction**
9.15 **Foot and mouth disease vaccines** F. Brown, *Wellcome Biotech, Pirbright, England*
10.00 **Rabies vaccines** J. Lécocq, *Transgène, Strasbourg, France*
10.45 **Coffee and exhibition viewing**

PLANT PROTECTION

11.15 **Baculoviruses** D. Bishop, *NERC Institute of Virology, Oxford, England*
12.00 **Frost protection** S. Lindow, *University of California, Berkeley, USA*
12.45 **Lunch and exhibition viewing**
2.15 **Viral resistance** D. Baulcombe, *AFRC Plant Breeding Institute, Cambridge, England*
3.00 **Herbicide resistance** G. Kishore, *Monsanto, St. Louis, USA*
3.45 **Bacterial and fungal resistance** R. Dixon, *Royal Holloway College, Surrey, England*
4.30 **Closing summary**

There will be ample opportunity for discussion within the programme as each formal presentation will be followed by a question period.

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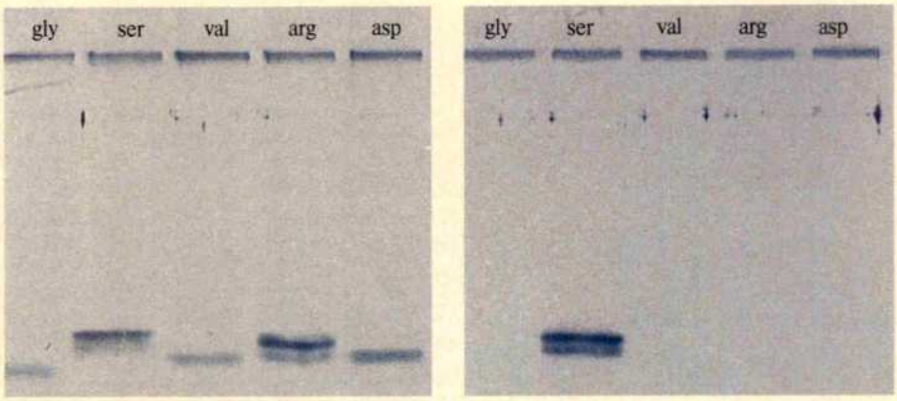

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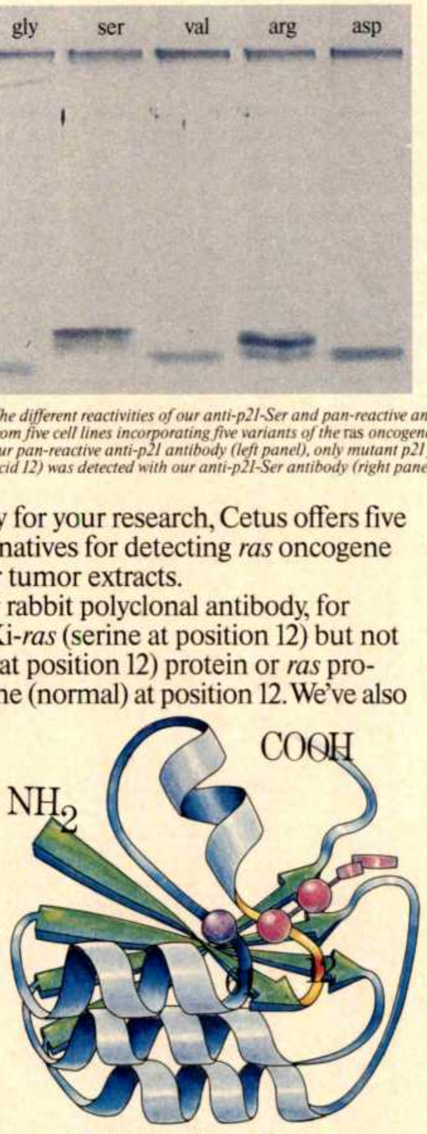

A model for the tertiary structure of the p21 protein. (By permission from F. McCormick, Cetus Corporation and Brian F. C. Clark, Aarhus University, Denmark.)

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nature

Too much panic over AIDS?

Most governments have reacted somehow to the AIDS epidemic, some regrettably illiberally but none has given epidemiology its due, in particular its capacity to rid us of uncertainties.

The British government, as if to deny the reputation of the British for understatement, has creditably done more than any other to take at their face value the pleas of knowledgeable people, last year's committee of the US national academies of science and of engineering, for example, that the first defence against AIDS should be public education. It has acted quickly and even radically, overturning conventions on the way.

The consequences have been twofold. First, British television programmes appear now to consist largely of solemn discussions of the mechanics of AIDS infection, which are complicated, and of the means of avoiding it, which are rudimentary but no more certain on that account. From a state of affairs in which, a few months ago, the word 'condom' was not useable in polite society, Britain has slipped effortlessly into a condition in which the public and commercial TV broadcasting organizations have cooperated in a demonstration (*inter alia*) of how don one of those disdained devices. Publications of all kinds appear to have risen to the challenge of saying what AIDS is all about, perhaps welcoming this implicit licence to tell something like it is. The second consequence should have been predicted; not merely are some people offended by the language of the message, but others are inclined to dispute its necessity. Newspapers tell true stories of physicians besieged by telephone enquirers. Others say that there is too much "panic" about AIDS. The origin of this sceptical reaction is merely that the British government, for all its willingness to spend money on public education, and for all the grave statements of public spokesmen that AIDS is a sombre threat to individual survival, has not said openly why it has (uncharacteristically) sanctioned all this spending: it believes (rightly) that it is squarely within the bounds of possibility that AIDS is a threat to the survival of whole populations, not just of individuals.

In cruel politics, it may be asking to much to ask that a government should be that explicit. It may be prudent to base policy on worst cases, but imprudent to say so in case the worst case does not materialize. If, in due course, it turns out that the transmission of the AIDS virus between heterosexuals is not facile enough to sustain the present epidemic, there will be many who will say, even on present evidence, that the British government has panicked needlessly, and should not therefore be trusted with other public duties, the defence of what used to be called the realm among them. It may therefore help the cause of rationality in what is necessarily a complicated matter that those concerned should come to grips with articles like that from Robert M. May and Roy M. Anderson on page 137. That is a technical account of what might theoretically be expected of the course of a sexually transmitted disease, which AIDS is, but all the more important for that reason.

Promiscuity

The chief inference from May and Anderson must be that, so far, little is known of the natural history of AIDS. For example, the incubation period, or the interval between infection and the appearance of symptoms of disease, appears at present to be 4 to 5 years, but that is chiefly because there has been no more time than that for the consequences of the infection to become apparent. Five years from now, the same authors may be saying that the incubation period, on the evidence then available, is 9 to 10 years (which might imply that those infected would have had twice as long to pass on the infection to others). Similarly, as May and Anderson make plain, there is no sure way of assessing the chance that an infected person will infect another as a consequence of a single act of sexual intercourse. The best they can do is to calculate the product of that probability with an arithmetical function which is essentially a measure of the contribution of promiscuity to the transmission of the AIDS virus.

Carriers

The conclusion is simple enough — promiscuous individuals are doubly ill-starred, because they are more likely to be carriers and also more likely to encounter carriers, so that their influence in the propagation of the epidemic is proportional to the square of the number of their sexual partners. All that is easily accepted, but has little bearing on the estimation of the crucial parameters — the incubation period, the duration of infectiousness of infected people, its distribution in time (episodic or otherwise?) and the likelihood of transmission (per encounter) between people of different sexes in different kinds of sexual encounters. In time, it could be that a better knowledge and understanding of the occurrence of AIDS in Africa could help pin down these crucial numbers. But that will not be easy, given the unwillingness of African governments to acknowledge that they (and we) have a problem to confront. The chance that useful epidemiogical data might be gathered from Haiti, supposed a few years ago to be the staging post between Africa and the United States, is similarly slim.

What, in these circumstances, should prudent governments attempt to do? (The word 'prudent' must be read both ways, as a means of safeguarding constituents and as a device for avoiding as much as possible of the political trouble that attends messengers carrying bad news that turns out to be wrong.) Surely the answer must be that epidemiology has a great deal to learn, and then to teach, about the nature and the dynamics of the present epidemic? The mindful British government which, earlier this month, endowed the Medical Research Council with the best part of £20 million to find a vaccine prophylactic, should now get out its bigger cheque-book to take on the more expensive, if less dramatic, work of understanding the mechanism by which AIDS is transmitted. How did so-and-so catch it, from whom, by what route, where did that infection come from and how common in the British population are the practices involved? Undertakings such as these are expensive because they entail talking to many more people than would be employed even on the most ambitious research programmes.

What goes for the British government applies to others. Unfortunately, most politicians appear to have settled for the quick fix, technological or otherwise. Both in India and in Belgium, it is now the practice that foreign students must be tested for AIDS on arrival. The information will of course be meaningless in respect of those who are infected, but in whom the process of sero-conversion (the appearance of antibodies) has not yet occurred; no doubt, the practice of this kind of ritual screening will persuade constituents that something is being done. Elsewhere, resources are being poured in two directions — the search for prophylaxis and, more immediately (and beneficently), the care of the sick. Nowhere is enough attention being paid to the need for an understanding and a quantitive description of the mechanism of this infection. The temptation to neglect this kind of work is understandable; epidemiology is slow, even slower than finding a vaccine. But on the assumption that AIDS will cast a shadow over at least several decades to come, is that not a case for starting soon? On the assumption that AIDS has presented us all with an unprecedented threat, to which countermeasures may not exist, may not the only visible remedy for a sense of panic be an understanding of the disease? ☐

Academic-wall writing

Discrimination between universities is beginning to bite in Britain. Where will it lead?

WITH the passage of time, the future pattern of British academic life becomes clearer, but not much brighter. Universities individually are beginning to come to grips with this year's allotment letters from the University Grants Committee (UGC), of which there is one universally good thing to be said this year — that universities will know where they stand in good time, and sufficiently far ahead of the financial academic year beginning on 1 August for them to hope to accommodate themselves to the changes under way. That, at least, is something to be grateful for. But the principal change now under way will please only some of UGC's constituents, those that have done relatively well in the system-wide competition for attention for having done well at research.

A year ago, in planning for the present academic year, UGC carried out a hasty assessment of the virtues of the separate institutions and shared out the funds at its disposal with the proviso that no single university's position would decline relative to the average decline by more than 1.5 per cent. The same "safety net", as the standard euphemism describes it, is also in operation this year. But the universities, many of whose employees are numerate, have used the interval to calculate that a 1.5 per cent relative cut, accumulated over five years, would amount to a disadvantage compared with the average of nearly 10 per cent. Because the bulk of university spending is on salaries and related benefits, it has suddenly become apparent to university administrators and even to their academic employees that it will not be long before there is a sharp division betwen some sheep and some goats; the establishments in between, whose nature has not yet been determined or clearly signalled, will be a dwindling group.

The way this year's cookie has crumbled is not very different from what happened a year ago. By no standards has any university been treated really generously; indeed, the funds available to UGC have increased by only 3.3 per cent in a year when the retail price index is likely to have done more damage than that to institutions' well-being, while most institutions are still at a loss to know how they will meet the increased pay bills due from last April. It is interesting that the places winning larger shares of the cake this year are, like last year's winners, some but not all of the newer creations — universities such as those of Bath, Warwick and York (with increases of recurrent grant of more than 6 per cent). Some (but again not all) of the large city universities have, like Oxbridge, also done better than the average, if modestly. At the other end of the spectrum are such places as different as the Manchester Business School and the University of East Anglia, which have done no better than the bare minimum. The Celtic fringe in Wales and Scotland has again come poorly out of the settlement, but piquantly, Heriot-Watt university, until recently a kind of polytechnic, has done better than its immediate neighbour Edinburgh (with increases of 4.3 per cent and 1.3 per cent respectively). The University of Wales Institute of Science and Technology, with 3.5 per cent, is better off than its older neighbour, the University College of Cardiff, with which it is in a prolonged merger dispute. There is plenty in all this for common-room heads to cluck about.

What cannot be dodged is the trend. Some institutions are on the way down and others may be staying afloat, or even marginally improving their positions. Moreover, as academic governing bodies throughout Britain know too well, even marginal changes can hugely determine their freedom of manoeuvre. A university losing real income may have to contemplate getting rid of people; one whose income rises will have the luxury of being able to employ young people with new ideas. So those that have done well in this year's competition may expect to do even better in future years. The Peter Principle ("To him who hath, etc....) applies. At this rate, it may not be very long before some British universities have become teaching institutions, of the kind that Mr Kenneth Baker, the Secretary of State for education and Science, was brooding about last week (see page 116). What remains to be determined is how the weakening institutions will respond to the adversity that afflicts them.

The first and natural reaction of such a place, and of those who work in it, is to protest that the process of enforced decline is unfair. So, in many senses, it is. There are, for example, well-attested anomalies in the way in which the funds have been shared. Many zealous academics have shown that UGC's ratings of departments accord with no known objective measure of their productivity in research. A more serious objection to the way that things have been done may be the tendency to take at their face value universities' own estimates of the cost of teaching their students. Small departments (and, therefore, small universities) get the short straws. Too little account may have been taken of industrial contracts, as distinct from public research grants, in assessing the non-teaching functions of universities. And it is a plain fact that even the least successful of the British universities competing for next year's funds are well supplied with creative and distinguished scholars who cry that insufficient account is taken of their contribution to the distinction of their institutions. The proper rejoinder has two parts. First, restrained commiseration; it may be bad luck to find oneself in a sinking ship, but is that necessarily a circumstance for which the occupants have no responsibility? Second, so what? Even if a substantial part of the British university system may in future have no great involvement in research, that does not mean that enterprising academics need be denied opportunities for scholarship. And who says that the education of the young can be left to the second-rate?

The plain truth is that the reshaping of the British university system now under way is necessary not only for reasons of economy but of scholarship as well. Too many parts of the system are too thinly supplied with talent to be creative on their own. It is more than twenty years since the Science Research Council, the predecessor of what is now the Science and Engineering Research Council, announced its firm intention to concentrate resources. Even earlier, UGC had made a few abortive attempts in that direction. But nothing much happened. The pity now is that the change has been enforced not by reason but by several years of public parsimony whose most conspicuous effects have been uneconomic savings, a waste of too many people and a general sense among academics that nothing is ever for the best. The research enterprise has been so damaged in this process that people have forgotten that what the system needs most of all is a mere handful of demonstrations that excellence still survives. Even the less lucky universities have a long-term interest in that state of affairs, for how else can their members and future students be persuaded to aspire to intellectual goals? Those which have have been lucky in the competition, and others, have a duty to demand that the more diverse system now in prospect provides opportunities for universities which may not be distinguished in research to improve.

Franco–US agreement on AIDS test within sight

- AIDS patent dispute near end?
- France and United States call truce

Washington

THE tangled legal wranglings between the US Department of Health and Human Services (HHS) and the French Institut Pasteur over the discovery of the virus that causes AIDS (acquired immune deficiency syndrome) and over the patent for a blood test that resulted from the discovery may be coming to an end.

A joint statement by Institut Pasteur and HHS released last week heaps glowing praise on both Robert Gallo of HHS's National Institutes of Health (NIH) and Luc Montagnier of the Institut Pasteur for their work on AIDS. But it then takes the curious tack of decrying "sensationalistic journalism" and condemning the "inaccuracies that have appeared recently and in the past describing the dispute between the two parties". The statement goes on to suggest that the legal dispute "involves highly technical issues of patent law" and does not diminish the contributions of Gallo and Montagnier.

The legal dispute concerns a patent awarded to Gallo for an antibody blood test for the AIDS virus. The Institut Pasteur has claimed that Montagnier should have received the blood test patent, as he had submitted a patent claim to the US Patent and Trademark Office some seven months before Gallo. The Patent Office has initiated an investigation that could lead to the patent reverting to Institut Pasteur.

Another dispute between the two institutions involves the sample of the AIDS virus that Montagnier gave to Gallo in 1983 for his research purposes only. The Institut Pasteur claims that Gallo used the French virus isolate to perfect his blood test, violating his agreement not to use the material for commercial purposes.

Both sides in the dispute are being extremely cautious in their public statements. Chuck Kline, a spokesman for HHS, says the language of the statement was only arrived at after multiple discussions between both sides' lawyers.

The joint statement takes pains to smooth over differences between the NIH and Institut Pasteur. It was issued by James Wyngaarden, director of NIH, and Raymond Dedonder, director of the Institut Pasteur and their respective lawyers.

The statement also appears to be the harbinger of an agreement, proclaiming that negotiations will lead to an "amicable settlement which will be honourable for all". A final line states that a settlement "would recognize the important contributions of Dr Gallo and his colleagues and Dr Montagnier and his colleagues leading to our understanding of AIDS and its diagnosis, and such a settlement should in no way be interpreted as providing either party with an advantage over the other party".

Both sides appear anxious to reach a settlement by the end of the month when French Prime Minister Jacques Chirac visits Washington. The settlement will probably include some mechanism for sharing financial benefits from patented blood tests, possibly through an international foundation first proposed by the United States last May (see *Nature* **321**,185;1986).

Montagnier (left) and Gallo. On the way to agreement?

Offending articles had made claims that "in the war against AIDS, scientific truth was among the first casualties" and suggested that Gallo clung to the idea that the AIDS virus — what Gallo then called HTLV-III and Montagnier called LAV — was more closely related to HTLV-I and II than it actually is. By doing so, Gallo indirectly caused many scientists to go off on the wrong track, the report claimed. Gallo had earlier discovered HTLV-I and II, two human retroviruses that apparently cause cancer.

The reports of Gallo's and Montagnier's work examined the similarities between the viral isolates used by them in developing their blood tests. Restriction maps have shown these two viruses to be indistinguishable, but sequence data show there are differences. Montagnier had given a sample of his virus to Gallo in September 1983, around the time Gallo says he succeeded in growing his own virus.

Last weeek Gallo and Mikulas Popovic were granted a patent for growing the AIDS virus in several different cell lines. This new patent covers virtually all cell lines in which the AIDS virus is currently grown.

Joseph Palca

Supreme Court reverses AIDS judgement

Washington

THE US Supreme Court, rejecting the argument of the Reagan administration, has ruled that a federal antidiscrimination law protecting handicapped workers extends to people with AIDS (acquired immune deficiency syndrome) and other contagious diseases. According to the Justice Department, an employer's fear of contagion, whether reasonable or not, was sufficient reason for firing Gene Arline, a Florida schoolteacher discharged from her job because she had tuberculosis. In a 7–2 decision, the court rejected this argument, ruling that employers receiving federal funds cannot deny jobs to people with contagious disease simply because of their own perceptions about the illness. Justice William Brennan, who wrote the opinion for the court, said "it would be unfair to allow an employer to seize upon the distinction between the effects of a disease on others and the effects of a disease on a patient and use that distinction to justify discriminatory treatment".

Reaction from the administration was muted. "The decision speaks for itself... we said what we had to say in our amicus [advice]", stated Justice Department spokeswoman Deborah Burston-Wade.

The ruling coincides with increasingly heated controversy over the rights of AIDS victims. One side argues that critical public health measures have been blocked by oversensitivity to civil liberties; the other side rejoins that protection of those liberties is the only way to get the cooperation needed to stem the AIDS epidemic.

Representative William Dannemeyer (Republican; California) attacked the public health community at a symposium on AIDS and employment held the day after the Arline decision. "Better that some people die of AIDS than that we transgress a group's civil rights", was his version of the US public health AIDS strategy. He claims that the male homosexual lobby is blocking measures such as mandatory blood tests and reporting names to the Public Health Service of those with positive tests.

Nan Hunter, of the American Civil Liberties Union (ACLU), argues that fear of discrimination and loss of rights is crippling the nation's response to AIDS. Cooperation in voluntary testing and safe sexual behaviour is essential, she says, but why be tested if it means losing fundamental rights? The objection is not to reporting numbers, Hunter says, but to the collection by the government of names and other information that can be used to discriminate against people in jobs, housing and medical insurance.

Nancy Heneson

Baker urges concentration of research in fewer universities

London

MR Kenneth Baker, the British Secretary of State for Education and Science, while still preoccupied with his running battle with schoolteachers over pay, is planning also to turn his attention to the administration of those parts of the research enterprise within his charge — academic research and the institutes of the four science-based research councils.

Further reorganization of the research council system is, however, unlikely. Mr Baker seems content with the present division of responsibility between the research councils, but is anxious that their work should be more pointed. One focus of concern is the extent to which research council funds are committed in advance by long-term obligations.

Mr Baker is less certain of the constitution of the Advisory Board for the Research Councils (ABRC), which gives him advice on the partition of the annual science budget among the research councils. One possibility is that the most interested parties, the research councils themselves, might not be directly represented on the council. Another is that the ABRC may be better provided with people able to monitor changing patterns of research at British institutions. Much will no doubt depend on the views of the ABRC chairman Sir David Phillips, of whom Mr Baker seems thoroughly to approve.

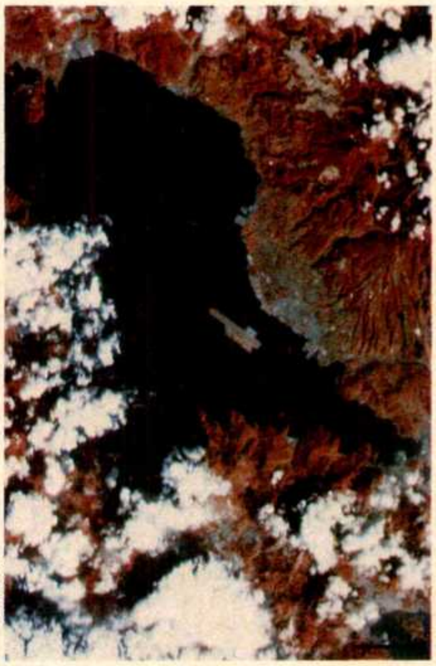
On the university front, the drive towards the concentration of research in a smaller number of universities or univerity departments, which has already influenced the allocations of funds to individual universities in the present academic year and, in a letter to universities last month, for the academic year beginning in August, will be encouraged. Last week, Mr Baker was even wondering aloud whether "some universities should become teaching institutions", with no substantial research activity.

Kenneth Baker sees tax reforms as a cure for the 'brain drain'.

Meanwhile, Mr Baker acknowledges that something must be done about the rate at which British scientists are being tempted abroad by better working conditions and, particularly, by better salaries. In the long run, he believes, the longstanding annual loss of something like 1,000 people a year, which may recently have increased, may require that attention should be given to radical changes in British tax arrangements to make them more competitive with those now in force in the United States under the provisions of last year's Tax Reform Act.

In the world of practical politics, however, Mr Baker says he will be looking at a variety of other measures. He hopes that the Royal Society's study of the loss of skilled people from Britain, due to be completed soon, will have some practical suggestions to make. He also wonders whether it may be necessary for some employers to take a leaf out of another US book, and to look to countries such as India for replacements for lost British academics.

John Maddox



NASDA

Tokyo

ONE of the first images taken by Japan's marine observation satellite Momo, launched on 19 February. This non-corrected false-colour image, taken by the satellite's multi-spectral electronic self-scanning radiometer on 23 February at about 10.50 a.m., is of Ohmura Bay off Kyushu (Nagasaki airport is visible in the bay linked to the mainland by a causeway). Blue and green represent bands 1 and 2 in the visible spectrum (0.51 – 0.59 µm and 0.61 – 0.69 µm, respectively), and red is band 4 in the near infrared (0.72 – 0.80 µm). The photograph spans about 20 km east to west and 36 km north to south. ☐

New civil service pay deal betters par

London

HIGH fliers in the British scientific civil service specializing in disciplines much in demand will reap substantial financial rewards in the next few years from the introduction of a radical new pay structure. As part of an offer by the British Treasury to the Institution of Professional Civil Servants (IPCS), there will be guaranteed increases of pay for all staff in the next two years, with the promise of further increases for people in fields with "recruitment and retention problems".

The pay offer covers IPCS staff working in the public service, not only in the civil service. IPCS is the negotiating body for some 60,000 scientists and technologists, many working for quasi-autonomous organizations such as the UK Atomic Energy Authority.

Those concerned will receive a pay increase on 1 April amounting to the percentage increase of the Retail Price Index in the year to the end of last month, expected to be about 3.9 per cent. There will be further pay increases in September and next April making a total estimated at 15 per cent. The new merit increases will also be effective from September.

One striking feature of the pay award is that it appears to breach the government's position that pay increases in the public sector should not exceed the rate of inflation. But the new structure has also been designed to reward individual performance and in such a way that merit awards are not uniformly spread over large diverse groups of scientific workers.

According to the Treasury, the intention is that the rewards of members of the scientific civil service should be determined by the performance of small, not large, groups in which they work and even on an individual basis, taking account of the skills employed as well as of "conditions in different parts of the country". The last of these provisions is in line with the British government's wish, put forcefully to a meeting of the National Economic Development Council last week, that pay bargaining should take account of the variation of living costs between different parts of Britain.

The scheme proposed for the scientific civil service aims to reward personal performance in ways that match those obtainable in industry. An essential part of the proposals is that there should be more effective individual job evaluation. The proposed deal, which will be effective until August 1989, will be discussed at a special conference of IPCS on 8 April.

Bill Johnstone

Japan's unpopular mental health laws to be revised at last

Tokyo

JAPAN's mental health laws are about to undergo their first revision in 22 years. The reforms, contained in a bill to be submitted to the Diet by mid-March, are a response to severe international criticism of Japan's mental health system.

The sorry state of Japan's mental hospitals first caught worldwide attention in 1984 when a scandal erupted at Utsunomiya Hospital. It was reported in the press that violence and confinement were regularly used to subdue patients, patients were forced to labour at a deep-freeze food factory owned by the hospital superintendent's family, others were made to act as assistant nurses and give injections to fellow patients. And, worst of all, two patients were allegedly beaten to death.

Representatives of the International Commission of Jurists (ICJ) and the International Commission of Health Professionals (ICHP) visited Japan on a fact-finding mission in May 1985 at the invitation of lawyer Etsuro Totsuka, one of Japan's foremost campaigners for mental health reform. A 94-page report released by the mission in September last year concluded that "the present structure and function of Japanese mental health services create conditions which are conducive to inappropriate forms of care and serious human rights violations". The major areas of concern identified by the mission were a lack of legal protection for patients, and a system of care characterized by a preponderance of long-term institutional treatment with little community treatment or rehabilitation. The commission recommended that an independent body be set up to review involuntary hospitalizations.

While the numbers of mental inpatients in countries such as Britain and the United States have been falling, Japan's inpatient population has grown more than twenty-fold over the past 30 years to 340,000, about 80 per cent of them involuntary admissions. Most of those confined against their will were committed by "consent" (*doinyuin*) under section 33 of the present mental health act. Consent is obtained not from the patient but from the family or a legal guardian. As there is still a considerable social stigma associated with mental illness in Japan, families are often only too willing to commit their mentally ill relatives. The only medical opinion required for such confinement is that of the superintendent of the hospital.

The main revisions to the law instituted by the Health and Welfare Ministry are: voluntary admissions will be encouraged but all of the clauses for compulsory admission will be retained in modified form (under the old law there was no clause for voluntary admission); prefectural review councils will be set up to hear patient's complaints and to screen involuntary admissions; local bodies and welfare organizations are urged to establish rehabilitation centres.

Etsuro Totsuka welcomes the revisions but still sees serious defects. "Consent hospitalization" is retained under a new name "protective hospitalization", the only change being that the medical opinion of a "designated doctor" rather than the hospital superintendent must be sought before committal and the patient's case will be periodically reviewed by the prefectural review council. But ministry officials expect that the designated doctor will come from the hospital in which the patient is to be hospitalized. Totsuka also questions the independence of a prefectural review council of three designated doctors, one lawyer, and one "man of knowledge and experience".

Professor Haruo Akimoto, former director of Tokyo Metropolitan Matsuzawa Hospital in Japan, also expresses doubts about the independence of the review council and he advocates inclusion of lay organizations such as the National Federation of Families of the Mentally Ill in Japan; Totsuka favours the British system of Mental Health Review Tribunals which consist of one doctor, one lawyer and one lay person.

A third serious defect is the lack of an equality clause to eliminate discrimination against the mentally handicapped. At present mental patients in Japan are barred from entering art museums or swimming pools; they cannot take up certain professions, such as hairdressing; and general rehabilitation and welfare services are not available to psychiatric patients. The Health and Welfare Ministry has asked the various ministries concerned to eliminate such discrimination, but no legislative action has been taken.

Finally, although there is a clause in the law calling for free, uncensored communication by patients with the outside world by letter, telephones are not mentioned. And there are no provisions in the law for meeting lawyers or independent doctors.

Both Totsuka and Akimoto welcome the clause calling for improved rehabilitation services. But they question whether the words will be backed up with government money. Akimoto hopes that "the new law is not just a gesture by the government intended to appease international criticism but that it will really assist rehabilitation and independence of the mentally ill". What is needed, he says, is reform of the hospitals, not just reform of the law.

David Swinbanks

NASA science head

Washington

LENNARD Fisk has been named to succeed Burton Edelson as head of the office of space science and applications at the National Aeronautics and Space Administration. Fisk is at present a professor of physics at the University of New Hampshire in Durham. Until his appointment was announced last month, he was chairman of the space science working group of the American Association of Universities and chairman of the National Academy of Sciences committee on solar and space physics.

Fisk's selection has been warmly received in the academic community. Thomas Donahue, chairman of the National Academy of Sciences space science board, calls Fisk "a card-carrying scientist" who will be able to work well with the space science community. His appointment takes effect on 6 April. J.P.

Death of Steve Prentis

London

STEVE Prentis, who died in a car crash on 27 February, had only recently become director of the Banbury Center and director of publications at the Cold Spring Harbor Laboratory, Long Island, where he was to be the editor of the journal *Genes and Development*, which was launched last week. Before moving to the United States, he had been based at the Cambridge offices of Elsevier in the United Kingdom, where he was initially editor of *Trends in Biochemical Sciences*, and then founding editor in turn of *Trends in Biotechnology* and *Trends in Genetics*. A second edition of his widely praised book, *Biotechnology: A New Industrial Revolution* has just been published. P.N.

Plea for return of exiles

London

THE University of Kabul has issued an appeal to former employees who have fled from Afghanistan to return home. Their jobs are still open, the appeal said, their reputations are still held in respect, the laboratories and libraries are awaiting them. The university will put at their disposal "every available opportunity" to prepare themselves for their return to teaching and to help them to carry out "better research".

The appeal, broadcast by Kabul radio, was presented as part of the current policy of national reconciliation. But the university also confessed its need of the emigrés. The lecturers now working there do not, it said, have the "knowledge, experience and energy" of those who went abroad. In fact, according to the Refugee Studies Programme of the University of Oxford, very few Afghan emigré academics have managed to find professional posts abroad, and most are still living in refugee camps. V.R.

Faisal international prizes in search of winners

Riyadh

THE short and somewhat shaky history of the King Faisal International Prizes was continued this week with the 1987 award ceremony in the Saudi-Arabian capital. Of the five annual prizes of £60,000 each, two of the three in Islamic/Arabic studies were not awarded for lack of nominations of sufficient quality. But there is no disputing the credentials of this year's winners of the medicine and science prizes, New Zealander Professor Barrie Jones of the International Centre for Eye Health at Moorfields Hospital in London and Sir Michael Atiyah, who is British and is professor of geometry at Oxford University. This year's only prize for Islamic/Arabic studies went to Sheikh Abubakar Mahmud Gumi, Grand Mufti of Nigeria for translating the Koran into Hausa.

Atiyah's prize, given for his work in the area between geometry and theoretical physics, is the first to be awarded for mathematics, one of the four subject areas for which the science prize is given in rotation. Chemistry was the area last year, and Dr Michael Berridge the winner. And in 1984 for physics, the prize was shared by Drs Gerd Binnig and Heinrich Roher, who won Nobel prizes last year. But in 1983, its first year, and in 1985, the science prize was not awarded, again "because nobody good enough was nominated", says Prince Khaled al Faisal, director general of the King Faisal Foundation which created the prize.

Saudi winners: Sir Michael Atiyah (left) and Dr Barrie Russell Jones.

The medicine prize, now in its sixth year, has always found a winner. It is awarded each year in a different area of medicine chosen to be of particular relevance to developing countries. Malaria, diarrhoeal disease and viral hepatitis have been among past topics. This year's subject, chosen by last year's jurors, was prevention of blindness. Jones is cited for his contributions to the prevention and treatment of trachoma and onchocerciasis.

In particular, his work established that chlamydial infections are responsible for trachoma. More recently, he has been pressing for the provision of simple programmes of teaching personal hygiene and health to rural communities in developing countries. That, he says, is a far more effective way to tackle blindness, 80 per cent of which could be simply prevented, than first to build a swanky specialist eye hospital, like the King Kalid Eye Hospital in Riyadh.

The annual process of finding prize-winners bears some resemblance to the Nobel process. With few exceptions, candidates have to be nominated by institutions: the Royal Society of London and the World Health Organization were among the nominees of this year's prizes. Relevant nominations are then refereed by anonymous experts in the subject area. The experts' reviews and the primary and other material are then sent to the relevant jury of "leading international scholars".

Foundation officials deny that sex, religion or race are factors in picking the winners, and jurors see no evidence of it either. On the other hand, of 14 science or medicine prize-winners so far, none has been female or Jewish. A sample of comparable size from other major prizes might also contain no women but would be unlikely not to include a Jew. **Peter Newmark**

Argentina to be sole user of nuclear dump

London

ARGENTINA'S proposed nuclear waste dump in the Sierra del Media area will not be 'internationalized', at least until the end of the century, according to Cesar Arias, a spokesman for the radiological and security protection office of the National Atomic Energy Agency (CNEA). Speaking on radio last month, he said that the decision whether or not to allow other countries to use the site is not due to be taken until the year 2000, and will then depend not on the CNEA but on the government of the day. Commenting on protests against the dump (to be sited 60 km from Gastre, in Chubut province), Arias said that the granite of the region made it one of the safest places possible. He dismissed the opposition as "normal", as, he said, nuclear energy had created among the general public "an aura of mystery, passion and even sex (*sic*)".

Hints that the dump would be made available to imported waste have been rife in Argentine political circles. One likely applicant to use the dump (if and when it becomes available) would be Brazil, where no less than 17 dumping sites (including some ocean sites) have been proposed and have been met with widespread opposition.

Vera Rich

Space museum exhibit 'escapes'

Washington

THE Smithsonian Institution's Air and Space Museum, one of the most visited of Washington's public museums, has come to the rescue of Magellan, the Venus radar mapping mission scheduled for launch in 1989. Magellan was to have been launched by the shuttle carrying a Centaur upper stage. But last June, NASA (the National Aeronautics and Space Administration) cancelled the shuttle/Centaur Program, forcing Magellan's managers to switch to IUS, the Inertial Upper Stage.

Because IUS is less powerful than the Centaur, weight had to be trimmed. A lighter upper-stage adapter was built, saving 127 kg, but this meant carrying out new structural load analyses. Typically, that would have been done with the prototype satellite, but that was already being used in the satellite construction. With no way to perform the tests, the launch date was in jeopardy.

Enter the museum's Voyager spacecraft exhibit. The bus on the satellite on display was close enough to the Magellan bus to be used in the tests. So, in January, workers removed the bus from the exhibit, sending it by truck to Martin Marietta's Denver plant where it will be modified to fit Magellan specifications.

MUSEUM OF PALAEONTOLOGY
That's nothing! We've had The President out on permanent loan for years...
AIR AND SPACE MUSEUM SAVES NASA

By using the Voyager bus from the museum, NASA saved approximately $1 million. But Magellan science manager Neil Nickle says that if the museum had not cooperated, no amount of money would have made it possible to construct another bus and still complete all tests by the scheduled April 1989 launch date.

Exhibits rescued from the Air and Space Museum have been useful for a real space mission before. Last November, the Air Force launched the Polar Beacon Experiment and Auroral Research satellite with parts from a Transit 5A satellite that had been hanging in the museum (see *Nature* **322**, 297; 1986). **Joseph Palca**

Money to flow again at NIH, but how long will it last?

Washington

THE National Institutes of Health (NIH) last week made a decision that will be music to their grant-holders' ears: money withheld from new grants awarded since the beginning of the year will be given back. But the respite may be brief. The plan to carry $334 million forward from the 1987 to the 1988 NIH budget, which initially prompted NIH's frugal action, is still on offer. Even if the money stays put, funds will be short in 1987 and could be extremely short in 1988.

The money to be handed back was part of the $334 million shift — known as extended availability — that would have lowered the 1987 budget to $5,850 million, even though Congress had already made its appropriation for 1987 at $6,184 million. For 1988, the Reagan administration requested $5,535 million, but with the money held over from 1987 the actual amount available for grants would be very close to the previous year's levels.

Congress must pass legislation in order to comply with the extended-availability scheme. So far that does not seem very likely. Senator Edward Kennedy has described the scheme as "robbing Peter to pay Paul". NIH director James Wyngaarden says the institutes have been withholding funds from all research grants since 1 January in order to spread the cuts over the months before 30 September, the end of the fiscal year. Individual institutes were responsible for decisions on how budgets were to be pared, but generally reductions were made across the board.

Some regarded the NIH decision to hold back funds as illegal. Approximately two dozen organizations, including the the Association of American Universities, were considering filing a lawsuit forcing NIH to release the funds. But legal action now seems unnecessary. On 24 February, NIH received instructions from the White House Office of Management and Budget (OMB) to release the money. The OMB made it clear that NIH should not "defer or otherwise restrict" currently available money until Congress gave its approval. But OMB spokesman Ed Dale says holding back funds did at first fit in with OMB's plans, although the cuts were not to be across the board.

It is still possible that Congress will agree to take the $334 million out of the 1987 budget. This could result in budgetary upheaval if all the savings had to come from money to be awarded in the final quarter of the year. But if the money is not shifted, the net result will be a 10 per cent drop in the NIH budget for 1988.

Joseph Palca

Brenner homes in on the human genome

DR Sydney Brenner, alternatively molecular biology's favourite son and *enfant terrible*, is to stake a personal claim in the sequencing of the human genome by recruiting a team of "six to twelve" people to carry out a mapping project.

Until recently director of the Laboratory of Molecular Biology of the Medical Research Council (MRC), and now director of the MRC's Molecular Genetics Unit at Cambridge, Brenner plans partly to finance the project with the £300,000 of research money that formed a part of his recently awarded Louis Jeantet Foundation Prize. Further funds, between £200,000 and £250,000 a year, are being provided by MRC's Cell Board as well as by other undisclosed sources.

The research unit will be based at the Babraham (near Cambridge) site of the Agricultural and Food Research Council's Institute of Animal Physiology (now rechristened the Institute of Animal Physiology and Genetics Research).

Brenner's objective is far from explicit, but appears to involve both the characterization of cells from different tissue types by the genes which are active in them and the use of techniques for copying messenger-RNA molecules into DNA so as to identify whole classes of cognate genes, which can then be placed on the human genome by molecular hybridization.

Brenner, who is a member of the US National Academy of Sciences committee on human genome sequencing, is robustly of the view that a successful project need not be as expensive as some of the first estimates of cost, exceeding $500 million (over 10 years). On the other hand, he says that novel developments in data handling and sorting may be necessary, which is why he is taking an interest in parallel-array processors.

John Maddox

Award for high-flyers at annual SDI black-tie banquet

Washington

BUILDING a strategic defence requires more than hardware, it needs dedicated people as well. That was the message from Lt-General James Abrahamson, director of the Strategic Defense Initiative (SDI) Office, as he helped last week to celebrate the second annual Strategic Defense Initiative Technical Achievements Awards Banquet sponsored by the American Defense Preparedness Association (ADPA).

An appreciative crowd packed the main ballroom at the Washington Hilton to see this year's winners receive their laurels. Seated among the military brass and defence contractors was last year's winner of the ADPA Strategic Defense Award, James Fletcher, administrator of the National Aeronautics and Space Administration. This year's award went to Edward Teller, associate director emeritus of Lawrence Livermore Laboratories, and Major-General Robert Rankine, director of space systems command, control and communications for the Air Force.

The evening was full of patriotic sentiment. The presentation of the colours was followed by the national anthem, and an invocation suggesting biblical precedents for a strong strategic defence. Dinner was steak and potatoes (filet mignon and Berny potato sticks); decaffeinated coffee was available for the fainthearted.

The awards for technical achievement went to the Delta 180 project, a successful attempt to intercept an orbiting, thrusting target by a thrusting interceptor from a starting separation of 200 kilometres. Runner-up awards went to a test last summer of a low-altitude rocket interceptor, and to Martin Marietta's rapid retargeting and precision pointing (R2P2) facility in Colorado.

Abrahamson was reportedly upset at the name "R2P2" because of its similarity to the robot character in the movie "Star Wars", but he managed a smile when the award was announced.

The laboratory award went to a team at the Air Force Geophysics Laboratory that developed a new long-wave infrared radar. Runners-up were an experiment to generate a multi-gigawatt burst of microwave radiation, and miniature valves to be used in kinetic-energy weapons.

Although the evening's emphasis was on scientific achievements spurred by SDI, Teller admitted that SDI work dealt with only "a few essentially new proposals". But Teller believes advances in technology for unmanned spacecraft necessitated by SDI will benefit the scientific community. He speculated on the day when SDI sensors could be trained on interesting astronomical events, such as the supernova in the Large Magellanic Cloud (see *Nature* **326,** 11; 1987 and see page 121 in this week's issue).

This was not a night for criticism of SDI, unless it was for Congress's failure to pursue the programme with enough vigour. Representative Jack Kemp (Republican, New York) got a standing ovation for his keynote speech urging deployment of SDI by 1993.

Joseph Palca

India's research council to undergo belated modernization

New Delhi

A REVAMPING of the Indian Council of Scientific and Industrial Research (CSIR) by an injection of young blood has been recommended by a committee set up 10 months ago by Mr Rajiv Gandhi, the prime minister. The committee has indicted the CSIR for its failure to help modernize Indian industry and has provided a new package of measures for invigorating its management and forging better links with industry.

Since he became prime minister, Gandhi has been critical of the functioning of CSIR, of which he is president. He feels that CSIR scientists have been reinventing the wheel rather than concentrating on mission-oriented projects or on technology to prepare India for the twenty-first century. A year ago, he showed his displeasure by replacing Dr S. Varadarajan, then director general of the CSIR, with Dr A.P. Mitra, a physicist who was previously director of the National Physical Laboratory. He then set up a seven-member panel headed by Mr Abid Hussian, a member of the Planning Commission, to review the work of the CSIR and to recommend changes to make it more responsive to the needs of industry.

The review is the fourth since CSIR was created, and the most critical. According to the committee, CSIR has lacked focus and direction and "has been unable to develop technologies which would meet even the most agonizing needs of our economy and society, let alone facilitate modernization". The major failure, it said, was the council's inability to transform scientific results in the laboratory into technology for industrial production.

In April 1986, CSIR had more than 1,000 projects under way. By putting emphasis on known products and processes, the council had failed to make a major impact. Declaring the CSIR system "unwieldy if not grotesque" the committee said that, by functioning like a department of government rather than as a society, it "has tended to foster hierarchy or bureaucracy and stifle creativity". The committee was also appalled by the "middle-age bulge"; most CSIR scientists are well over 40 years of age and have stayed simply because there has been no mechanism for firing the more unproductive scientists.

Lack of team work has also been criticized by the committee, which found the CSIR to be a "loose federation of different laboratories rather than a cohesive system working for concrete goals and specified objectives". The committee observed that the council and the industrial sector have acquired distorted images of each other; industry believes that CSIR is incapable of useful and timely research, while CSIR believes that "industries prefer the soft option of importing proven technology".

The committee's chief recommendations are aimed at bridging this gap. It has proposed that six of the nine members of the CSIR governing body should be from outside the CSIR. The chairman of the governing body should be an eminent professional scientist rather than, as at present, the CSIR director-general, and the governing body would relinquish its present role of administrative watchdog and focus more on formulating policy guidelines. The research councils of each laboratory would similarly be reconstituted with members representing industry and consultancy organizations.

First, get rid of the dead wood...
FORESTRY RESEARCH DEPT.

Some of the recommendations relate to personnel. The director's tenure would be fixed at six years, after which he would return to research work. The strength of non-scientific staff would be reduced by half in three years and promotion would no longer be automatic. The committee has asked CSIR to get rid of unproductive scientists by encouraging voluntary retirement on generous terms. To bring in new blood, CSIR should hire good scientists on six-year contracts "at salaries 50 per cent more than the usual". A scheme of visiting scientists and another of distinguished fellows have been suggested to improve the climate for research. And there should be an institute for retraining and reorienting the entire CSIR fraternity "which has been allowed to be denuded without being recharged".

All CSIR laboratories have been asked to take up research projects identified by users and to work together with private industry. The linkage would go beyond the customer–contractor relationship "through exchange of personnel and equipment". The link with public-sector companies should be ensured by associating CSIR technologists with the acquisition by industry of imported technology "right from the beginning".

CSIR has also been asked to earn a third of its annual expenditure through consultancy to industry or government: the remainder would continue to come from public funds. In turn, industry has been asked to pay 0.75 per cent of the value of its output. This money could be used by industry to sponsor projects in CSIR laboratories.

The committee did take note of the achievements of the CSIR. Industrial production based on technology licensed by CSIR is now about £250 million. Some 50,000 tractors and a range of modern pesticides have been manufactured using CSIR expertise, and novel catalysts and petroleum-refining processes developed at CSIR have enabled India to emerge as a licensor in this high-technology field.

K.S.Jayaraman

Respite for French researchers

London

RECRUITMENT of young French researchers, frozen since last June, is to start again in all disciplines but biology, the new science minister, Jacques Valade, has announced. His decision to renew appointments for life to France's leading research council, the Centre National de la Recherche Scientifique (CNRS), is seen by applicants for research posts as a major victory in their long-standing struggle with the government.

The move not only affects those eligible for the jobs available in 1987. It also clarifies the position of the 285 young scientists deemed eligible last year, who were briefly promised jobs only to find their appointments invalidated by government decree (see *Nature* 324, 9, 1986). The 285, after extensive lobbying by their pressure group, the "Collectif des Admissibles", have had their one-year temporary contracts extended for life. In acting to restore the posts of the 1986 *admissibles*, Valade has managed to break the legal and political deadlock in the CNRS caused by the decision of his predecessor, Alain Devaquet, to overrule the 1986 appointments and to overhaul the appointment procedure.

Some problems still remain, however. Appointments in the life sciences remain blocked because of alleged 'irregularities' concerning the representation of the medical teachers' union on appointment committees. A decision is expected soon from the French high court. The *admissibles* have vowed to continue their campaign for permanent posts until the problems in biology are satisfactorily resolved, but, in the words of one CNRS spokesman, "at least everyone can breathe again".

Neil Crombie

The route to arms control without the hardware

Washington

As current talks in Geneva on strategic weapons in Europe emphasize, arms control negotiations tend to focus on force reduction. But a new report, published under the auspices of the American Academy of Arts and Sciences and the Cornell University Peace Studies Program, suggests more attention must be paid to the "nonweapons aspects of national security policy".

The report examines the viability of present command, control, communications and intelligence (C^3I) systems. If both the United States and the Soviet Union expect deterrence to remain the basis for preventing nuclear war, then command systems must be sufficiently secure to make the ability to retaliate credible. But the report suggests that vulnerabilities of the current command system make it "likely to be severely degraded, if not collapse" following a major nuclear attack.

Several factors have conspired to make stability harder to achieve during a crisis. Paul Bracken, professor of public policy at Yale University and one of the report's authors, says the complexity of the strategic battleground requires intelligent computer systems to make it comprehensible. But as financial markets' response to computerized trading practices have shown, such systems can precipitate large responses, especially as human judgement is eased out of the loop. One recommendation of the report is to improve communications systems for the president and his top advisers. Other recommendations include additional systems to assure communications within the military hierarchy and field forces, especially submarines equipped with nuclear weapons.

Hillman Dickinson, former director of command, control and communications systems for the joint chiefs of staff and another author of the report, says modernization of US C^3I should have the highest priority in the strategic budget.

Cornell physics professor Kurt Gottfried and Richard Garwin, an IBM fellow at the Thomas J. Watson Research Center, organized the report. Garwin suggests that issues relating to C^3I have not generated much interest because "thinking doesn't cost money", so there is no profit motive driving such discussions. The initial report is aimed at generating public and congressional interest in the topic. The report's suggestion of a 1,500-mile interdiction zone for nuclear weapons around Washington and Moscow may capture the public imagination. Such a zone would give Washington a warning time of 15 minutes of a nuclear attack, much more than is now available.

Joseph Palca

The report, "Crisis Stability and Nuclear War", is to be published in expanded form later this year by Oxford University Press.

Conflicting signals from LMC supernova

London

A JAPANESE/US collaborative team has announced the detection of a burst of neutrinos from the recent supernova explosion, SN 1987a, in the Large Magellanic Cloud (LMC). But the timing of the burst conflicts with the detection reported earlier by the Italian/Soviet collaboration working at the Mont Blanc neutrino observatory.

The neutrinos were revealed as 'electron events' — Cerenkov radiation from high-energy electrons — in the Kamiokande II experiment, a nucleon-decay and solar neutrino observatory comprising 3,000 tonnes of water, located 1,000 metres underground in the Kamioka mine, western Japan.

At 7:35 Universal Time (UT) on 23 February, about 18 hours before the first optical sighting of the supernova, the Kamioka detector recorded a burst of 12 electron events in the space of 13 seconds. The electron energies ranged from 7.5 to 36 MeV, and the first two events pointed back to the LMC. The remaining ten events are consistent with an isotropic velocity distribution, but this could indicate that these events represent the passage of antineutrinos — which scatter off protons — rather than neutrinos. Although detailed statistics are not yet available, a member of the collaborative team said that the neutrino signal was "exceedingly clean". In the experiment's previous 1½ years of operation, no unexplained electron events with energies above 20 MeV had been detected, yet the burst contained three such events.

The Mont Blanc observatory detected its burst of five events about 4½ hours before the Kamioka detection. If both detectors were recording neutrinos from SN 1987a, the bursts should have arrived simultaneously; the discrepancy thus calls into question the reality of one or both detections. Unfortunately, a third observatory which might have detected the supernova neutrinos, at the Homestake mine in South Dakota, did not record any events at all — a result which is nevertheless consistent with the Kamioka detection because of the much smaller size of the Homestake detector (see p.135). Laura Garwin

Oftel moves to help Mercury

London

FOREIGN telecommunication monopolies needing a British link to their international networks will be required to allocate contracts to the two competing carriers in the United Kingdom in the same proportions as their overseas traffic.

This new ruling is that of Oftel (Office of Telecommunications), the watchdog of the British telecommunications industry which has become concerned that the new company Mercury, which is competing with the giant British Telecom, once a state-owned monopoly, is given a fair chance to compete.

Mercury, a wholly owned subsidiary of the international group Cable & Wireless, was given its final operating licence in 1984. In the same year, British Telecom was 'privatized', allowing it to compete more effectively overseas and to forge partnerships outside the United Kingdom. But Oftel worried that the previous contacts in overseas carriage enjoyed by British Telecom because of its earlier monopoly, could freeze out Mercury from the international scene.

The ruling on overseas monopolies is in the form of a 'Determination' issued by Professor Bryan Carsberg, director-general of Oftel, to ensure "that overseas monopolist operators cannot use the competition between British Telecom and Mercury to gain an unfair advantage".

The terms of the operating licence of both the British carriers require that they agree to some formula to monitor international traffic. In the absence of an agreed code, Oftel has imposed its own.

The new rules are a further attempt to liberalize the UK telecommunications market. Mercury, however, will be protected from further competition — by government decree — until the end of the decade.

Oftel is little concerned, at this stage, about agreements between British operators and those overseas carriers with indigenous competing telecommunications companies. Nor are they concerned if such overseas partnerships have the effect of reducing costs and increasing the efficiency of the services. While a substantial proportion of British Telecom's revenue comes from overseas traffic, it should remain unaffected if the corporation continues to generate future traffic in the same proportions as it has in the past.

Many European telephone companies still enjoy a monopoly and would be scrutinized under this new ruling. The United States has liberalized its market while Japan is in the process of deregulation. Their telephone companies are likely to be least affected. **Bill Johnstone**

Creationism in Queensland

Sir—The report (*Nature* **321,** 89; 1985) by Tony Thulborn on the evolution controversy as it affects secondary biology teaching in Queensland is fundamentally in error. The chief error is the statement that the teaching of creationism "is officially included in the school science curriculum". The truth is that creationism was deleted from the Queensland science syllabus in 1983. A teacher wishing to include creationism, or any other deviation from the syllabus, in the high-school biology curriculum must receive approval for the work programme from the Board of Secondary School Studies acting on recommendations by the District and State Review Panels. In other words, not only is creationism *not* included in the official science curriculum but its inclusion as a local variation must satisfy two screenings before receiving the board's approval.

Thulborn's statement that Queensland science teachers "are now obliged to present the conflicting arguments for creation and catastrophism" thus bears no relation to the prescribed curriculum. His statement is a highly tendentious interpretation of remarks by the Minister for Education, Mr Lin Powell, concerning "balance" in the teaching of evolution. Mr Powell has spoken for himself in the letters column of this journal (*Nature* **324,** 204; 1986).

Building on this fundamental error, Thulborn describes a school-room crisis. He reports the formation of the Australian Association for the Protection of Evolution at a rally called to combat the furious onslaught of creationism. The alarming threat is heightened by reporting the decision taken by the association to assemble "survival kits" for teachers facing the creationist scourge. This crisis exists entirely in the imaginations of Thulborn and a handful of his associates. The survival kits have never been issued, no doubt for the good reason that they are irrelevant to the life of science instructors.

Hiram Caton

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Thulborn replies—My report about creationism in Queensland was neither erroneous nor fanciful.

An instruction to include creationism in the science syllabus was first issued to Queensland school principals on 30 November 1981 by the then Minister for Education, Mr William Gunn. In November 1983, as Hiram Caton observes, the Board of Secondary School Studies released a draft syllabus (biology) which made no mention of creation. Nevertheless, the present Minister for Education, Mr Lin Powell, insists that teachers should still heed the 1981 instruction. In 1984 he tabled that instruction (*sic*) in the Queensland State Parliament, in response to questions about the teaching of creationism in state schools (*Hansard,* 10 April 1984). One member of parliament noted that the minister "failed to confirm or deny if biology teachers were being forced to teach creationism" (*Sydney Morning Herald,* 12 May 1984). In 1985, Powell said that "there would be no change in the secondary science syllabus, and the 1981 recommendations still stood" (*Brisbane Courier-Mail,* 30 April 1985). And in a published letter he has stated that the 1981 directive was "to ensure that students are exposed to both creationism and evoution" (*Brisbane Courier-Mail,* 13 May 1985). It is therefore legitimate to conclude that Powell, in his official capacity as Queensland's Minister for Education, has instructed teachers to include creationism in the school science syllabus.

Caton describes elaborate procedures to monitor the content of the science syllabus. Those safeguards certainly exist, though they are sometimes circumvented or ignored. In fact they have been most blatantly abused by the minister himself: Powell's instructions about "balanced" presentation have been addressed directly to school principals, thus side-stepping the approved procedures whereby the science syllabus is constructed and maintained by the Board of Secondary School Studies. In any case, it is obvious that existing safeguards do not exclude creationism from science classes.

In May 1984, an informed source in the Queensland Education Department revealed that only half of the science curriculum was prescribed by the department. "The remainder is determined by each school, which has to submit a school work program to the Board of Secondary School Studies. That board has been partial to proposals to teach creationism" (*Sydney Morning Herald* 12 May 1984).

In the same newspaper article, a senior science teacher commented that "creationists now have an open hand to teach it [creationism] with the official sanction of the Queensland Government and many are doing just that. The obvious next step is for creationism to become compulsory teaching in Queensland schools, and we are perilously close to that now." In addition, a lecturer at the Brisbane College of Advanced Education confirmed that several schools were known to teach creationism, identifying one of them by name. Last year, one science teacher recounted his experience when he refused to introduce creationism in his biology classes: those classes were promptly handed over to a teacher trained in Home Economics (*The Skeptic,* November 1986, p.20). He also mentioned that one of his colleagues was being harassed because his teaching of ancient history conflicted with literal interpretation of the Bible. In short, a few teachers are fundamentalists who will teach creationism regardless of any official prohibitions; and other teachers have been encouraged, pressured or plainly instructed to bring creationism into their science classes.

On 10 July 1986, the executive committee of the Australian Academy of Science issued a statement condemning the teaching of creationism in school science courses. That statement makes particular reference to Queensland. Similar action has been taken, or is being considered, by other scientific, educational and religious bodies throughout Australia. Recently the Continuing Education Unit at Queensland University offered a short course specifically designed to help local schoolteachers to deal with evolution and creationism in their classes; more than 70 people enrolled.

The publication of a popular book titled *Creationism, An Australian Perspective* (edited by M. Bridgstock and K. Smith, Australian Skeptics, Melbourne) has been resoundingly successful: the first two editions were sold out in a matter of weeks, and the third has just been released. The book is proving particularly useful to the many teachers who are understandably perplexed by the whole issue. I am pleased to report that the Australian Association for the Protection of Evolution made useful contributions to these and several other ventures.

Tony Thulborn

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Research assistants

Sir—I note that you continue to publish letters on the "plight of British postdocs". I write now to suggest a comparatively simple step that would at least remove one irritation. I refer to the use of the word "assistant". While it is not unreasonable to advertise for "research assistants" at the grade of postgraduate, it seems both insulting and inaccurate to refer to "postdoctoral research assistants". Surely those advertising are seeking people who should contribute originality to the solution to a problem and are not mere assistants.

We suggest that postdoctorals should in future be referred to as research scientists, research biochemists or research fellows.

P.N. Campbell

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UK citations

Sir—In view of the publicity given to the perceived decline of British basic science (see for example the leading article 'British science over the hill', *Nature* **323**, 655; 1986) the recently published list of the most-cited 1984 life sciences articles from the Institute of Scientific Information in Philadelphia (*Current Contents, Life Sciences*, **29**, No. 49, 3–17; 1986) is of interest.

Of the 102 most-cited papers, Dr Eugene Garfield has found that 61 of the institutions in which the research work was carried out are located in the United States and 13 are in the United Kingdom. Countries with which the United Kingdom has recently been unfavourably compared in fact produce relatively fewer scientific papers in this 'immediate high-impact' category; only seven of the institutions are in France, four in Japan and one in West Germany.

The comparatively strong presence of the United Kingdom in this latest record of trends in the life sciences seems not merely attributable to excellence in one or two areas, as the highly cited British papers covered very different topics such as growth factors and oncogenes, intracellular messengers, ion channels and secretion, transition metals in disease, antibodies in AIDS, renal transplantation, human complement genes and leishmaniasis. In view of the recent attacks on the British university system, it may also be worth pointing out that most of the highly cited UK papers came from research groups working in universities.

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On the defence

Sir—I have a certain sympathy for Alvin Weinberg's preference (*Nature* **324**, 610; 1986) for a "defence-dominated" regime with vastly reduced nuclear arsenals. But I am not optimistic about its ultimate feasibility, as more than just scientific questions are involved. The United States and the Soviet Union would also have to trust each other enough to agree to release each other from the mutual hostage relationship of nuclear deterrence.

The Reagan administration's current unilateral and aggressive pursuit of strategic defences — the Strategic Defense Initiative (SDI) — seems almost designed to prevent this. The Soviets clearly view SDI as an attempt by the United States to achieve a first-strike capability and will redouble their efforts to maintain their deterrent force, they are hardly likely to respond by reducing their nuclear arsenals. A defence-dominated world cannot be imposed unilaterally but can arise only through mutual agreement after a long process of disarmament and the development of some degree of trust among all the nuclear powers.

Weinberg may be right to complain that the Cornell survey of the National Academy of Sciences members' views on strategic defence failed to offer the possibility of a defence-dominated world. Taking these political considerations into account, however, I doubt whether many members of the academy would have given it high prospects.

Mark Goodman

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Sir—I read Alvin Weinberg's letter (*Nature* **324**, 610; 1986) with care but find his argument for supporting the Strategic Defense Initiative (SDI) difficult to follow. He poses the question "...if arms control leads to a mutually agreed reduction in the offensive threat to, say, a few hundred offensive missiles, can a defensive system be effective against this much lower threat? The answer to this question is probably yes... ." The essential word in this quotation is "if". Can one reasonably expect the Soviet Union to agree to reduction in missiles to a level that renders its offensive capacity negligible in the face of a deployed anti-missile system — in effect, unilaterally to disarm?

The Soviet Union is likely instead to seek parity, not in nuclear warheads or launchers, but in *deliverable* warheads. Thus any success in developing SDI by the United States will almost certainly be matched by an increase in nuclear missiles by the Soviet Union. It is true that this situation may be modified if the Soviet Union itself develops and deploys an anti-missile system on the scale of SDI but the inevitable temptation then for the United States will surely be to increase its own weapons to ensure that it maintains what it considers to be an adequate deterrence.

The history of warfare has been a constant competition between offensive weapons and defensive systems with developments in one serving as a stimulus for the other. Why should we suppose that the response to SDI should be any different?

D.W. Mason

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Sir—Alvin Weinberg's letter (*Nature* **324**, 610; 1986), criticizing the Cornell Survey of the opinions of National Academy members of the Strategic Defense Initiative (SDI), has a basic flaw that invalidates his argument. His criticism is based on the expectation that SDI may be effective against an attack by a few hundred missiles rather than the several thousand now possessed by each side and that missile reduction should therefore be sought. Weinberg proposes that critics of SDI should work towards such a reduction *and* for SDI as a means of stabilizing arms reduction.

The Reykjavik meeting brings out the inconsistencies of Weinberg's argument. The prospect of a substantial missile reduction collapsed over Soviet concern with SDI. That concern is probably based on Weinberg's thesis that SDI will be effective against the reduced missile threat rather than the present Soviet missile force so that a large reduction would leave the Soviet Union at a serious disadvantage.

A second thesis for Soviet concern would be an inability to respond to a US first strike. To many of us, such an action may not seem likely, but the situation could appear different to Soviet leaders. The repeated pronouncements of US presidents, from Truman on, on the use of nuclear weapons, and the repeated rejection by the United States of a 'no first strike' policy, would make the Soviet Union oppose an SDI programme that would prevent a Soviet response to a US attack.

Consequently, SDI and nuclear missile reduction are not incompatible, as Reykjavik demonstrated. Thus, Weinberg's criticism of the Cornell survey is not justified, and his questioning of the scientists motives is inappropriate.

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Shades of 1968

Sir—Under the headline "Shades of 1968?" (*Nature* **324**, 500; 1986) one reads that "just why General de Gaulle's government took such fright...has never been crystal clear".

Some clarity may be provided by a slightly different statement of the facts.

The students did not protest against a "modest package of educational reforms". They were chanting slogans such as *"l'imagination au pouvoir"* a vague claim — although unreasonable — if ever there was one.

Then the whole country came to a stop when strikes paralysed industry, public services and the administration. Elections were called, a new government was formed and for the first time Edgar Faure took charge of the Ministry of Education. He then had a new law voted that led to what might appear as sweeping changes rather than "anodyne reform".

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Archaeological split

SIR—Much has been made of the 'great success' that attended the breakaway 'World Archaeological Congress' held at Southampton in September 1986. According to information one of us has received from the chairman of that meeting, "over 1,000 people registered from about 100 countries".

It is important to place these figures in perspective. The attendance of 1,000 contrasts sharply with the 2,000–3,000 that the organizers originally expected. Moreover, we would remind our colleagues that at the IXth Congress of the International Union of Prehistoric and Protohistoric Sciences (IUPPS), held at Nice from 13 to 18 September 1976, which, in accordance with the constitution of IUPPS, was open to scientists from all countries, 3,127 participants from 94 countries were present.

So, at the price of holding a meeting by sacrificing a major principle, the organizers gained five or six countries, lost 2,000 participants and did grievous harm to British and world archaeology. Add to that the unprecedented split in British and world archaeology, the wedge driven between colleague and colleague, the stimulus given to a major cleavage and politicization of world sciences ... and we cannot see that all this adds up to 'a great success'.

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Throwing stones?

SIR—Your correspondent Vera Rich never misses a chance to revel in *schadenfreude* at any misfortune that befalls the Soviet Union, which she immediately attributes to inadequate planning or ability to cope by the authorities. The latest outburst was her report (*Nature* **325**, 188; 1987) of the spell of very cold weather that hit most of Europe between 11 and 16 January.

I have visited the Soviet Union three times during very cold winters and was impressed by the way buildings (including private homes) were heated, streets and railways kept free of snow. All forms of public transport ran efficiently, and diesel vehicles had preheated fuel and oil.

I was in England during this year's cold spell and was appalled by the inability of the authorities there to cope. Many roads, including some motorways, were closed, there was chaos on the railways and old people were dying of hypothermia in their homes, all this at temperatures 30° C above what the Russians were experiencing. The military managed to overturn a vehicle carrying a nuclear weapon, an event that might well have developed into a catastrophe of Chernobyl proportions.

Maybe there is some Vera Rich in the Soviet Union churning out pejorative copy about the inability of the capitalist authorities to cope with a spell of only slightly cold weather, and their callous disregard of the plight of their citizens. But what such a correspondent would write could genuinely be classified as *pravda*.

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Space colonization

SIR—Von R. Eshleman's reservations about the near-term feasibility of space colonization deserve attention (*Nature* **324**, 115; 1986). Colonization historically has been successful when existing technology permitted the migration of substantial numbers of people into large uncultivated areas that were suitable for agriculture. Conversely, experience has shown that the exploitation of a hostile environment for commercial or scientific purposes does not result in colonization. For example, the mapping of the ocean floor and offshore oil drilling have not resulted in any noticeable increase in the population of the oceans.

The rest of our Solar System is not suitable for agriculture. If the colonization of 'space' is judged to be a worthwhile goal, one way to proceed would be to use remote sensing devices to identify Earth-like planets in other Solar Systems. Once an Earth-like environment has been identified, it would be appropriate to initiate plans for colonization. An attempt to colonize the rest of our Solar System would probably be as cost effective and as successful as the European colonization of Greenland from AD 986 to 1599.

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More vaccine needed

SIR—The article by Tim Beardsley describing the formation and goals of the Task Force on Hepatitis B Immunization (*Nature* **324**, 399; 1986) may inadvertently give the impression that we intend to achieve widespread hepatitis-B virus (HBV) immunization in high prevalence regions of the world solely through use of the vaccine produced by the Cheil Sugar Co. in Seoul, Korea.

That company deserves credit for having produced a pure and potent vaccine that can be manufactured at relatively low cost. Moreover, because of the generosity and humanitarian spirit of Mr B.C. Lee, chairman of Cheil's parent company, SAMSUNG, it is the first to commit itself to providing vaccine for public sector mass immunization programmes at $1.00 per dose, the maximum cost for a vaccine to be considered for inclusion in mass immunization programmes.

But the actual need for HBV vaccine throughout the world will be several hundred million doses a year, so it is clearly necessary for many producers to share responsibility for meeting these needs.

The Task Force is therefore in communication with all potential manufacturers of HBV vaccine, from whom we hope to be able to elicit similar commitments.

The Task Force favours widespread use of *all* safe, effective and affordable vaccines so that hepatocellular carcinoma and cirrhosis in high prevalence regions of the world can be controlled.

ALFRED M. PRINCE
(Chairman)

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Valuable libraries

SIR—As users of the human chromosome-specific libraries prepared by the Los Alamos and Livermore National Laboratories of the Department of Energy and distributed by the American Type Culture Collection, we wish to refute the suggestion that these libraries are not of value to molecular geneticists (*Nature* **323**, 660; 1986). In our experience these libraries represent a valuable and freely available resource. Their "deficiencies" reflect a state of the art problem. There is no doubt in our minds about the "biological talent" of the scientists involved, nor of the usefulness of the libraries.

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Eiffel Tower threatens top astronomy

A French company's understandable pride in having built a conspicuous structure in Paris nearly a century ago may persuade it to ruin optical astronomy for several years to come.

Herstmonceux

WHEN the 4.2-m William Herschel Telescope comes into operation in the Canary Islands this summer, it will be the largest telescope to which British and Netherlands astronomers have access, and the third largest in the world. Far from city lights, it will be able to see the faintest galaxies. That, at least, was the original plan. But while people are putting the final touches to this fine instrument, the directors of the Eiffel Tower Company are contemplating a project that could seriously hinder its use, not to mention that of all other optical telescopes.

The intention of the *Société Nouvelle d'Exploitation de la Tour Eiffel* is to mark the centenary in 1989 of the Eiffel Tower with a display of space art. Having created a monument visible from all Paris, the company in its egotism wishes to create another visible from all the world. Unbelievably, this project is associated with the French Centre National d'Etudes Spatiales and the European Space Agency, even though the scientists in these organizations do not support it.

The Eiffel Tower Company has selected two proposals by competition. The front-runner is called the Light Ring, which would be launched to an altitude of 800 km in a 90-min orbit. At launch, the ring-structure of 2-cm tubes connecting 100 Mylar spheres each 6 m in diameter would be compacted into a cubic metre weighing 500 kg. After it was deployed, the heat of the Sun would evaporate 4 kg of water inside the structure, inflating it to a diameter of 24 km. It would appear as bright as 100 stars of magnitude +1 and would cover a patch of sky 0.5 degrees in diameter — the size of the Moon.

If this Light Ring passes over an optical telescope that is looking up, the observation will be ruined because light from the bright ring will totally swamp the natural starlight. If the telescope is feeding a sensitive amplified detector like the Image Photon Counting System (IPCS) in use on La Palma, the detector will be permanently destroyed, along with the phosphor and cathode of its sensitive image tube which is its main component. There are at present about ten suitable image tubes left for the IPCS in the world and each is literally priceless. It is not of course the only detector at risk. The area of sky swept out by the satellite in one pass over a telescope means that there is a one per cent chance that the telescope will accidentally view the Light Ring on each pass — a virtual certainty that an accidental viewing would happen within a year unless precautions were taken.

The Light Ring would be visible from most countries of the world — that is its point. The whole of a summer's night and half a typical winter's night would be at risk.

The orbits of large, low-density structures are very uncertain because of the effects of atmospheric drag, and the pressure of solar radiation, including particle pressure which depends on solar activity. Errors of 15 min in an orbit predicted three weeks in advance are at present not unusual. The orbit of the Light Ring will be more uncertain because it is much larger than anything launched so far. Astronomers would have to invent ways to protect equipment from it all the time.

The proposers estimate that, with a 1989 launch date, but depending on sunspot activity, the Light Ring will last for three years before it is burnt up in the atmosphere.

If, on further study, the Light Ring poses insuperable technical difficulties, the Eiffel Tower Company proposes to select an alternative 'space art' project called Arsat. A curved reflecting sail would be launched into an 11-h orbit and would focus the Sun into a cross with a bright centre, projected on the Earth over a 3,000–5,000-km region.

From within the cross for three-quarters of an hour, Arsat would be visible as the brightest star, which would flash with a period of a few seconds as the sail slowly spins in its own plane, rotating the cross over a continent. Because Arsat would be focused, the satellite could be very bright. The project author calculates that it could perhaps be brighter than the Full Moon, flooding the sky with light. It would make it virtually impossible to carry out observations on faint stars while the satellite was above the horizon.

The costs of these two space art objects are in the range $1–10 million, within the possible budget for a global celebration.

A more bizarre proposal than space art has come from the Celestis Corporation in Florida. It is to launch in reflective orbiting mausoleums the cremated remains of 15,000 individuals (compacted by a patent process to 3 cm^3 each), in orbits that would last "for eternity". At $50 million per launch, this is a profitable proposition.

The US Department of Transportation (which is responsible for promoting the commercial space industry) has approved the Celestis Corporation's morbid project, which now depends only on market demand.

The effect of innumerable satellites visible to the naked eye orbiting over observatories would be to sweep bright trail after bright trail through a telescope's field. Already, virtually every deep photograph taken with the UK Schmidt Telescope in Australia over a 6 degree area of the sky is spoiled by an artificial satellite trail, those of the first and last two hours of the night having several trails. Even for the narrower half-degree field of a conventional telescope, the Celestis Corporation proposal has the potential to interfere with every image.

What of the future? Pierre Comte, the Arsat proposer, is an artist with *Leonardo*, a magazine devoted to the interface between art, science and technology. He writes: "Each time an event of worldwide significance takes place (Olympic games, international expositions, anniversaries important to our planet), it would be possible to announce it, to celebrate it by means of the presence of the Arsat satellites parading on the vault of heaven: a mobile star of extreme brightness, a group of stars forming an ephemeral and symbolic constellation . . . I simply tried to create an object that was beautiful, with pure lines and a dreamlike vocation: a star in the sky, but one born of human hands, a mad dream..."

Dreamlike or visionary this may, in a sense, be. But what a nightmare it is to anticipate a sky filled with orbiting trademarks or political symbols, offensive billboards perpetually overhead, from which there is no escape on Earth.

To counteract the space funerals, the International Astronomical Union in 1985 maintained that "no group has the right to change the Earth's environment in any significant way without full international study and agreement". These quiet and reasonable words do not give full force to the passionate feelings of astronomers on this issue. The noble, aesthetic aims of the space artists have been perverted in the egocentric proposals under consideration by the Eiffel Tower Company: the Celestis Corporation's materialistic proposals do not even have aesthetic virtue. The world's astronomers hope that these projects and others like them will be cancelled.

Paul Murdin

Organic geochemistry

Bigger and better hopanoids

Guy Ourisson

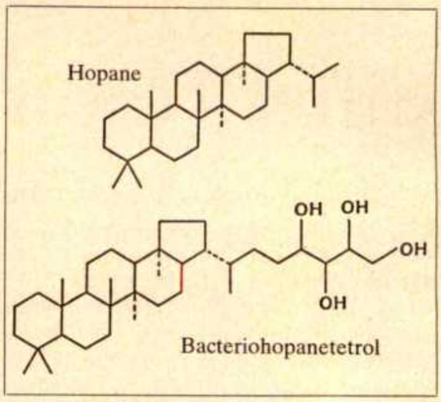
THE isolation of intact bacteriohopanetetrol, a precursor of most fossil hopanoids (microbial lipids), is reported by B. Mycke and colleagues on page 179 of this issue[1]. The authors achieved this result by the rhodium-catalysed hydrogenolysis of kerogen (the insoluble organic fraction) from a 50-million-year-old shale. Their finding demonstrates that intact biological structures can be preserved in the high-molecular-weight organic components of sediments.

The Eocene shale studied by Mycke *et al.* is from Messel, near Darmstadt, a well-known palaeontological site. As well as very delicate fossils of snakes, fishes, crocodiles and other tropical vertebrates, Messel has also yielded an impressive list of molecular fossils, in particular of 'geohopanoids'. The discovery of bacteriohopanetetrol, derived from the C_{30} triterpene skeleton of hopane by a five-carbon extension (see figure), increases by one the long list of these modified but recognizable derivatives of hopane found in sediments. Why is it remarkable that bacteriohopanetetrol has now been added to the list? To answer this question, I will first recount briefly the unusual story of hopanoids.

The first C_{29} and C_{30} hopane derivatives were discovered about 30 years ago in the resin of tropical trees Dipterocarpaceae, and slightly more recently in ferns. In 1966, Hills and Whitehead described[2] the isolation of the first fossil hopanoid, a crystalline hydrocarbon obtained by careful fractionation of the high-boiling fraction of a Nigerian petroleum.

The advent of gas chromatography coupled with mass spectrometry allowed consistent, unambiguous syntheses, and over the next 10 years it became clear that geohopanoids are extremely varied, quite abundant and ubiquitous[3]. They have been found in all shales, clays, limestones, coals and petroleums that are down to at least 500 million years old. They comprise stereoisomers (useful in evaluation of the maturation of sediments), aromatic or ethylenic hydrocarbons, alcohols, acids, methylketones, aldehydes and thiophenes. The C_{30} pentacyclic skeleton of geohopanoids can be epimerized, rearranged, degraded to tetracyclic structures or cyclized further to hexacyclic structures. Most importantly, the skeleton can be extended by a straight chain of up to five carbon atoms, and maybe even a few more[4]. The abundance of geohopanoids is staggering: overall, their total mass is about 10^{11-12} tons, which is about as much or more than the total mass of organic carbon in all living organisms.

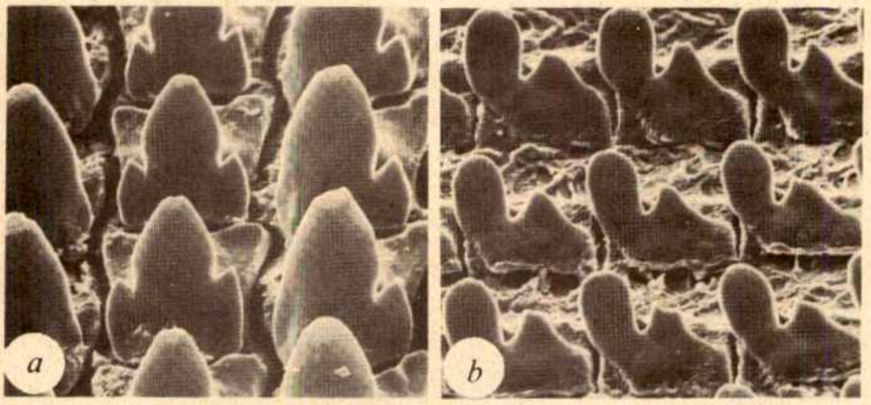
The accumulation and ubiquity of geohopanoids demanded more than an anecdotal explanation in term of ferns or of odd tropical trees. An explanation was also required for the C_{31}–C_{35} geohopanoids, for which no reasonable precursor was known: intuitively, one could accept that maturation in sediments could be accompanied by degradation or by rearrangements, but not by the stepwise addition of one to five carbon atoms as a straight chain.



The discovery[5] of C_{30} hopanoids in a few blue-green algae and bacteria suggested that the origin of these geohopanoids was microbiological, and this explanation became highly probable once bacteriohopanetetrol had been isolated from an *Acetobacter*[6] and from a thermophilic bacterium[7]. This hopanoid was initially believed to be a 'special' metabolite, responsible in each case for a particular property of the microorganism producing it. But it rapidly became clear that it is only one of a large family of biohopanoids, and that these are extremely widespread, although not ubiquitous, among the most diverse bacteria[8].

Since then, thanks mostly to the work of M. Rohmer's group[9], the family of bacterial biohopanoids has been extended to include polyols, aminopolyols, aminocyclitol ethers, carbamoyl derivatives and even bacteriohopanetetrol-peptides and a C-nucleoside, 30-adenosyl-hopane. These complex hopanoids form a family of bacterial lipids of general significance, having in bacterial membranes the same structural role as cholesterol in eukaryotic membranes, and formed from squalene by the action of enzymes, the squalene–hopane cyclases, which are probably phylogenetic precursors of the epoxysqualene cyclases of plants, fungi or vertebrates[10,11]. Bacterial hopanoids are "microbial lipids betrayed by their fossils"[12]. The youngest, least-matured sediments contain the least-modified structures; it is therefore not surprising that bacteriohopanetetrol has been isolated from a recent soil and from bacterial mats. It is, however, rather reactive, which makes its isolation in a pure state difficult, and explains its many transformations in the inorganic matrix of the sediments.

The isolation of intact bacteriohopanetetrol in a 50-million-year-old shale by



AN almost ubiquitous molluscan feature is the radula, a toothed chitinous mouth structure used to pulverize food. The lower surface consists of many rows of minute teeth; the rasping oscillatory motion of the array of teeth against the floor of the mouth is sufficient to break up the chosen morsel, which can then be ingested. The electron micrographs show details of radular teeth of the land snail *Euryptyxis fragosa* (*a*, central and lateral teeth; *b*, marginal teeth, × 600), and are taken from a paper by Peter B. Mordan in the *Bulletin of the British Museum of Natural History (Zoology)* 50, 207–271; 1986. This paper describes the southern Arabian Enidae, a group of non-marine molluscs, the detailed structure of the radula being an important diagnostic device in the taxonomic classification. The land snails are known to show a high degree of temporal distributional stability, and a comprehensive classification should eventually be useful in the biogeographical analysis of a region that, because it is located where three geographical zones coincide, has many population discontinuities. □

Mycke *et al.*[1] thus shows that complex, intrinsically reactive molecules can be efficiently protected by their inclusion (in this case as ethers) in the inert matrix of a heterogeneous polymer such as kerogen. It is remarkable that in the same Messel kerogen other highly informative fragments — the C_{40} bis-phytanyl chains characteristic of the lipids of methanogenic archaebacteria — had been isolated earlier.

It may seem surprising that the complex molecules isolated from sediments are often bacterial membrane constituents. But this certainly results from the fact that they are mostly derived from the terpene metabolism, and are therefore highly branched and biodegraded only with difficulty. In contrast, the major constituents of the microorganisms, proteins, reserve lipids and carbohydrates, are built to have a fast turnover: they are easily metabolized, either internally or, after death, by saprophytic microorganisms. It can now be assumed that the insoluble organic matter of other sediments (such as lignites and coals) is largely derived from the most resistant constituents of the bacterial populations involved in the first stages of compaction, and that selective reactions will lead to the isolation of recognizable parts of structures. □

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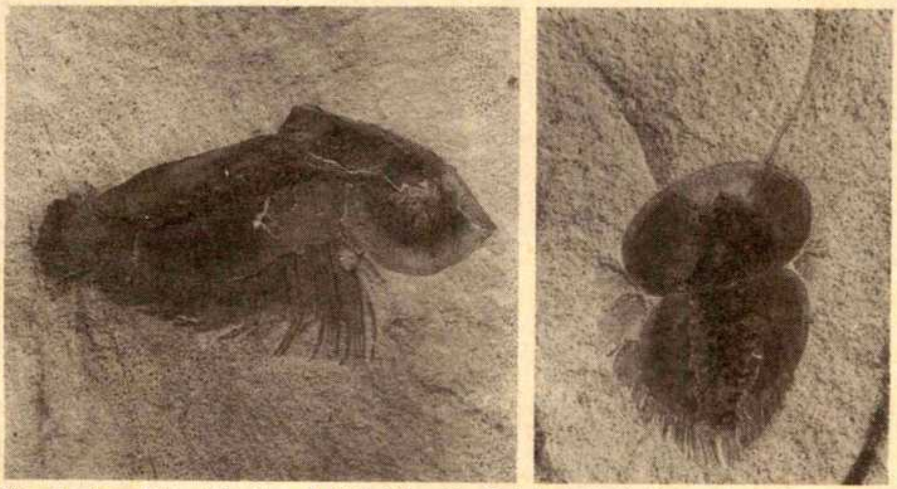
Palaeontology

Life in the Cambrian seas

Desmond Collins

OUR knowledge and percepetion of Cambrian life has changed dramatically in recent years. One major contribution to this change was the re-study[1] of the Burgess shale fossils by H. B. Whittington which, besides confirming the predominance of arthropods, revealed many animals of unknown affinities. Now, another corner of the veil obscuring our view of Cambrian life has been lifted. On page 181 of this issue[2], S. Conway Morris and colleagues describe a soft-bodied Burgess shale-like fauna from the Lower Cambrian of Greenland. The fossils they describe are a small collection of rather nondescript arthropods. Fortunately, as in Salome's dance, what lifting the veil suggests is more interesting than the view itself.

The Greenland arthropods lived 10 million years or more before the animals of the Burgess shale, and so demonstrate the evolutionary stability of the fauna. They also lived thousands of kilometres away from the Burgess shale basin (now in British Columbia), showing that the fauna was geographically widespread. The Greenland fossils described in this issue thus confirm the view of Whittington and colleagues that the animals of the Burgess shale were typical of life in Middle Cambrian seas, and also demonstrate that such animals were probably typical of Lower Cambrian seas.



Naraoia, from Mount Stephen, British Columbia. Soft-bodied trilobites such as *Naraoia* and *Tegopelte* have now been found in Lower Cambrian faunas in Greenland, Idaho and China, and in Middle Cambrian faunas in British Columbia and Utah. (Compare with Fig. 2*e* of Conway Morris *et al.*[2] on page 182 of this issue.) The specimen on the left is 35 mm long; that on the right is 30 mm from tail to tip of head, excluding antennae.

Specifically, the Greenland fossils repeat the preponderance of arthropods in the Burgess shale, particularly the dominance of soft-bodied arthropods over arthropods, such as most trilobites, with mineralized skeletons[3]. An additional fact, not emphasized in ref. 2, is the common occurrence of soft-bodied trilobites in this and other soft-bodied Cambrian faunas. The largest Greenland arthropod is similar to the soft-bodied trilobite *Tegopelte* from the Burgess shale, and another (Fig. 2*e* of ref. 2) looks very like *Naraoia* (see figure in this article), a soft-bodied trilobite found in the Burgess shale and in the underlying *Glossopleura* fauna[4] of British Columbia, in the Middle Cambrian of Utah and the late Lower Cambrian of Idaho[5], and in the early Lower Cambrian of China[6]. Soft-bodied trilobites, along with the other soft-bodied arthropods, seem to have been common animals in the sea for much of the Lower and Middle Cambrian epochs.

Sepkoski's statistical analysis[7] of the origin and diversification of marine families in the Palaeozoic complements these changes in our perception of Cambrian life. Sepkoski observes that the pattern of diversification in the Lower and Middle Cambrian is appropriate for animals evolving to establish and fill ecological niches in the world's oceans. These animals had broad habitat and food requirements. Once competition for living space began in earnest in the Upper Cambrian, the generalized animals of the 'Cambrian fauna' declined and the more specialized animals of the succeeding 'Palaeozoic fauna' expanded. This interpretation is certainly consistent with the generalized organization of the Burgess shale arthropods, many of which do not fit any modern category, and with the great number of animals of unknown affinities in the Cambrian.

The study of Cambrian soft-bodied fossil faunas such as those in Greenland, British Columbia and elsewhere thus reveals the broadest range of 'body plans' that developed on Earth. Moreover, such fossils provide an exceptional insight into the evolution of life forms in conditions of minimal competition. □

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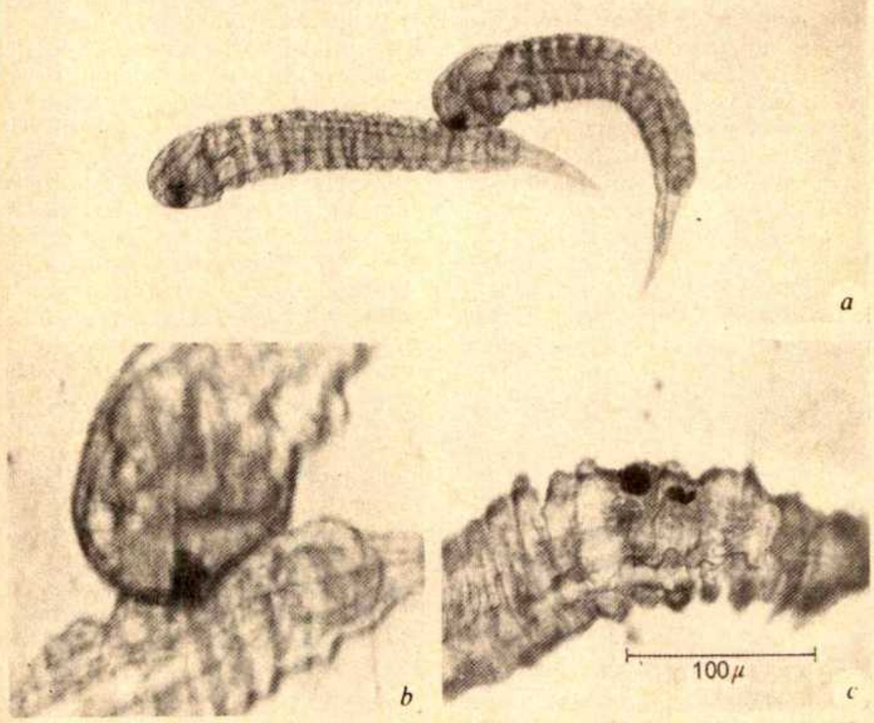
Evolutionary biology

Murderous mandibles and black holes in hymenopteran wasps

Paul H. Harvey and Linda Partridge

FOR the most part, biologists view adaptive evolutionary change as reversible. For example, when dark moths were selectively favoured through camouflage against a background of sooty tree trunks, it came as no surprise that lighter forms became more frequent again after the introduction of smoke-control measures. But there are instances of apparently irreversible evolution in which, following reversion to an ancestral environment, ancestral phenotypes do not re-evolve. For example, the transition from a mating system that entails high frequencies of inbreeding to one of outbreeding may be straightforward. But in an outbreeding population of diploid organisms, deleterious recessive genes can accumulate. The transition back to inbreeding now involves a selective disadvantage because the progeny of incestuous parents is more likely to receive a double dose of the deleterious recessive alleles. Charnov and Bull[1] have recently described such cases of irreversible evolution as involving 'absorbing states' which might be defined as evolutionary routes that, once taken, preclude return. (We would have preferred the expression 'black holes' so that an eminent astronomer could contribute for once to evolution theory.) Recent work suggests[2] that a particularly gruesome absorbing state is involved in the distribution of gregariousness among species of parasitic wasps.

Apanteles, a braconid hymenopteran genus, contains more than 1,300 species which typically lay their eggs in lepidopteran caterpillars. The young feed on their host and emerge as it dies. Of 276 species for which data have been brought together by le Masurier[3], more than half are solitary with a single young feeding on its host. Among the gregarious species, "there is a broad peak of brood sizes from 12–26, with some much larger broods (up to 1,200), but relatively few species with brood sizes in the range 2–11 larvae per host"[2]. Why are there so few species with 2–11 young per host? Among gregarious species, there is a strong correlation between host size and the total volume of the parasitoid brood. The absence of gregarious species with brood sizes 2–11 cannot be explained by a dearth of suitably sized hosts. These are available, but they are parasitized by solitary species. The answer seems to be more intriguing and involves mortal combat. Many of the solitary species possess long piercing mandibles that are used to kill their fellow parasites in the same host. Salt[4] describes the course of these fatal encounters — however many parasites are laid, just one survives.



Living first-instar larvae of the wasp *Nemeritis canescens*. *a*, *b*, Larvae fighting each other 72 hours after parasitization; *c*, melanized mandible marks, fifth day after parasitization. Magnification of *b* and *c* is about 3.5 times that of *a*. (From ref. 4, courtesy of Dr R. Fisher.)

Godfray[2] has now produced population-genetic models that throw light on the evolutionary dynamics of the process. Under what conditions would a gene for fighting invade a gregarious species? And when would a gene for cooperation spread in a population of fighters? If fighters are present in a brood, they kill all non-fighters and then attack each other until only one individual is left. Genes for fighting can spread whenever competition for resources within the host gets too intense, but mutant genes for tolerance in a population of fighters are soon done to death. In the simplest models, the actual condition for genes for tolerance invading a populations of fighters involves an Allee effect (individuals must have a higher fitness as one of a group than when alone). Once genes for fighting have spread, there is selection on mothers to reduce their clutch size to a single egg. The mandibles still provide a selective advantage because they can be used to kill the offspring of other mothers laying in the same host. And the production of solitary aggressive young can remain an evolutionary stable strategy even when the environment changes to favour larger optimal parental brood sizes. There is no point in a parent laying the larger optimal clutch size unless genes for tolerance have invaded the fighting populations and this, we have seen, is unlikely. Hence, argues Godfray, the strange bimodal distribution of clutch size in *Apanteles* is to be expected.

If Godfray is right, there should be solitary species that parasitize large hosts that might have been expected to provide nourishment for several parasites. Such species exist; when the parasites of large-bodied hosts are compared, the biomass of gregarious species per host is more than ten times larger than that for solitary species[3]. Also in agreement with Godfray's explanation is the finding that only solitary parasite species inhabit small-bodied hosts, whereas both solitary and gregarious species occupy larger hosts[3]. Once aggressive behaviour has evolved, which is likely to occur whenever parental optimal clutch sizes are reduced to two or three, it is very unlikely that tolerance, and hence gregariousness, will evolve again in that evolutionary line[2].

Analagous models for the evolution of siblicide in parasitic hymenoptera may also be applied to nestling birds. According to Godfray's work, the conditions for the spread of fighting and tolerant genes are the same for diploid and haplodiploid species. Siblicide is known in birds, but why is it not as common as in the parasitic hymenoptera? The occurrence of superparasitism in the hymenoptera may be one reason — when more than one mother typically lays her brood on the same host, the likelihood of a gene for tolerance spreading is reduced. Another contributing factor may be the typical avian hierarchy in size and fighting ability which facilitates the spread of tolerance[2].

Bull and Charnov[1] cite other examples of absorbing states, for example, once dioecy has evolved "ever-increasing specializations of males and females lead to the point at which both phenotypes cannot successfully function in the same individual". Herein lies one generality which the authors derive from their study of irreversible evolution. They point to the principle of accommodation. Once a state has evolved, the genome may progressively accommodate that state until reversible evolution is unlikely. The accumulation of deleterious recessive genes in outbreeding populations reduces the probability that inbreeding can be reinstated, and the production of aggressive larvae in small clutches of parasitic hymenopteran larvae may effectively close the evolutionary route back to gregariousness.

The examples of irreversible evolution so far known involve some fundamentally different underlying processes. At present, it does not seem possible to predict the circumstances under which other cases might be found. However, it is now evident that evolutionary biologists must take their own version of black holes seriously — absorbing states in evolution exist, and they may crop up in the most unexpected places. ☐

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DNA structure

Tailors or machine operators?

Maxim Frank-Kamenetskii

PROGRESS is not necessarily contingent on the sophistication of equipment. People engaged in the DNA business have long been learning this lesson. Expensive ultracentrifuges have been ousted by trivial gel slabs as separators of DNA molecules. Now, apparently, it is the turn of nuclear magnetic resonance spectrometers and X-ray diffractometers to be rendered superfluous. These sophisticated machines are most efficient in studying the atomic structure of small DNA pieces under special conditions. But they are practically useless when one wishes to know what spatial structures the various parts of the natural DNA molecule adopt under conditions close to those inside the living cell. Among other things, the structure of a given DNA stretch may depend on ambient conditions including the surrounding sequence of nucleotides. This has now been convincingly demonstrated by K.M. Sullivan and D.M. Lilley (*Cell* **47**, 817–827; 1986).

Sullivan and Lilley used a very simple enzymatic method to detect cruciform extrusion in palindromic (invertedly repeated) regions in their DNA preparations. It has previously been shown that the two loops of a cruciform are preferentially attacked by the S_1 endonuclease, a process easily detected by gel electrophoresis. Sullivan and Lilley studied cruciform extrusion under superhelical stress in two different plasmids, one of which carries the ColE1 palindrome in its natural surroundings and the other carries the so-called bke palindrome inserted at the *Bam*HI restriction endonuclease site.

These two plasmids exhibit strikingly different behaviour with respect to cruciform extrusion as a function of ionic strength and temperature. This difference could arise from the differences in sequence between the two palindromes. Sullivan and Lilley tailored their plasmids so that the bke palindrome was flanked by ColE1 and the ColE1 palindrome was sewn into the *Bam*HI site. What did they observe? The answer was unambiguous: the behaviour depends on a nucleotide sequence that flanks the palindrome rather than on the palindrome itself. The anomalous behaviour of palindromes in the ColE1 surroundings probably results from the fact that the region is highly enriched in AT pairs.

The paper by Sullivan and Lilley is the most recent, but by no means the only, demonstration of the great advantages of the methods of genetic engineering in studying DNA structural polymorphism. Many people are altering natural DNA pieces, tailoring specially designed sequences, incorporating them into plasmids and probing the plasmids with enzymes and chemicals to investigate which alternative structures other than the canonical B-form various DNA sections can adopt under different conditions. The important lesson to be learnt from the work of Sullivan and Lilley is that the result of such probing may depend not only on the region itself but on the surrounding sequences as well. ☐

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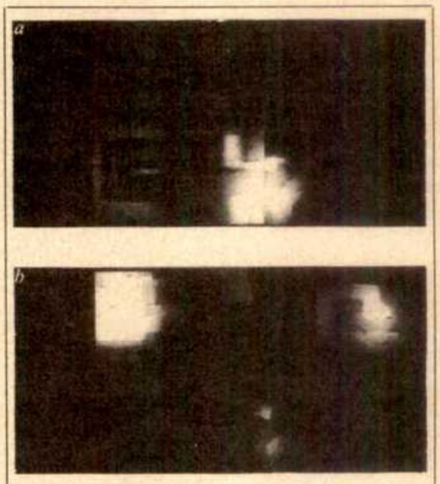
Joule heat comes into its own

ON THE heels of the tunnelling electron microscope, and on the principle that it should be possible to construct a microscope of some kind around any microscopic phenomenon, two people at IBM's T.J. Watson Research Center at Yorktown Heights, New York, have now announced the development of the Joule displacement microscope, a device that can follow the flow of electric current in a microcircuit by the detection of the surface displacement brought about by Joule heating of the underlying semiconductors (Martin, Y. & Wickramasinghe, H.K. *Appl. Phys. Lett.* 50, 167; 1987).



Micrographs of the inverting gate. Field of view, 600×360 μm. *a*, Photodisplacement in phase with input clock; *b*, photodisplacement by π out of phase with input clock. (Courtesy of Dr Y. Martin.)

The essence of the technique is the use of a heterodyne laser interferometer, essentially a Michelson interferometer using two laser beams mismatched in frequency and which, by the use of the semiconductor surface as one of two reflectors, is less sensitive to drift of the optical path-length.

By way of demonstration, the system is used to follow changes in the topography of the surface of a semiconductor circuit (which required that the focused laser spot should be scanned across the surface), taken to be a commercially available invertor gate operating at 10 kilohertz. Because the heterodyne laser makes it possible to measure separately the in-phase and out-of-phase signals from the surface, it is possible to indentify separately the pieces of the circuit which are expanded at different points in the cycle. The two photographs above show the images obtained when the phase angles differ by 90 degrees.

The obvious applications of the technique are to the study of the performance of developed microcircuits and the search for faults in prototype devices. ☐

Neurobiology

An eye on ancient circuits

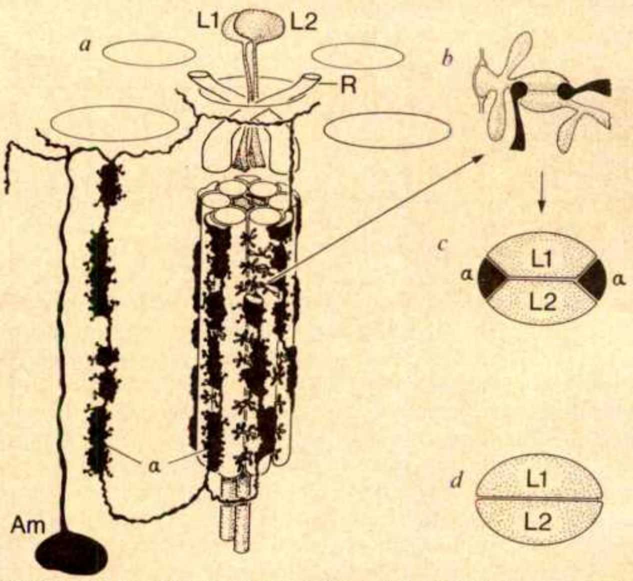
Melody V.S. Siegler

EVERY nervous system has an evolutionary past and is very much answerable to it — such is the message of three recent papers that examine the rise and fall of neural circuits in flies[1], firebrats[2] and assorted other invertebrates[3]. In invertebrates, it is possible to recognize similarities between individually identifiable neurons, not only in different ganglia of the same species but also across widely separated families and orders. The authors of the three papers choose neurons for comparative study that are similar enough to be recognizable as homologues but different enough to be interesting. They conclude that these neural elements are essentially conserved but adapted and modified as new behaviours evolve.

Shaw and Meinertzhagen[1] ask how nervous systems might change during evolution to produce innovations in an animal's behaviour. They suggest two possibilities: either new types of neurons emerge that connect with and modify existing circuitry; or synaptic connectivity among existing neurons alters. They have studied the fine structure of the lamina, the outermost layer of the optic lobe, where photoreceptor axons synapse on precisely ordered arrays of identifiable postsynaptic neurons, in a range of dipterans, from ancient craneflies to modern houseflies and blowflies (see figure).

The lamina, along with the rest of the optic neuropile, has already been studied in exhaustive detail[4] but never before, it seems, with an eye to at least 300 million years of evolutionary past. Shaw and Meinertzhagen conclude that all flies' eyes are not alike. They could recognize laminar neurons of more than a dozen uniquely identifiable types in each of the 14 species they examined. Moreover, the types seem to be homologous across the families, indicating a widespread conservation of neuronal form over time. What has changed is synaptic form, as revealed unequivocally in serial-section electron microscopy. Specializations of synaptic ultrastructure occur progressively in the phylogenetic sequence. These include elaboration of the presynaptic dense bars, imputed to be instrumental in the release of transmitter, and of the capitate projections of glial cells, possibly involved in membrane recycling.

Against this background of gradual synaptic change, there is one striking alteration in connectivity. Whereas in lower flies each photoreceptor terminal synapses with two postsynaptic elements to form a 'dyad', in the higher flies dyads are replaced by the well-known dipteran 'tetrads', with an extra two postsynaptic profiles provided by pre-existing neurons (see figure). The tetradic arrangement, the authors conjecture, may be part of a local feedback loop that enhances the resolution of transient stimuli, important in the execution of the rapid turning movements in flight that are characteristic of houseflies and blowflies.



a, A lamina optic cartridge of the housefly, of the relatively recent family Muscidae, Diptera. Six photoreceptor axon terminals (R) converge around two large, monopolar neurons (L1 and L2) that run at the central axis of the cylindrical assembly. Small processes from L1 and L2 take part postsynaptically as a pair at many small, tetrad synapses on the surface of each presynaptic R terminal, together with two extensions that normally come from α processes (black) of lamina amacrine neurons (Am). *b*, Higher magnification of the postsynaptic processes at the postsynaptic site. *c*, Section through the tetrad of postsynaptic processes at the postsynaptic site. *d*, Dyadic arrangement of the processes in some older families of Diptera. (Modified from ref. 2, courtesy of Ian Meinertzhagen.)

Among the most primitive of living insects are the wingless firebrats. Edwards and Reddy[2] have used these domesticated and cosmopolitan relatives of silverfish, denizens of bakeries and boilerhouses, to delve into the evolutionary past of insect behaviour. In particular, they ask how the wingless Devonian progenitors of modern insects evaded their terrestrial predators, spiders and scorpions. They find that firebrats have elements that seem to be the prototypes of an escape system elaborated in more advanced modern insects, cockroaches and crickets: a pair of specialized abdominal appendages, or cerci, that bear wind-sensitive sensilla, and several associated giant interneurons. The cell bodies, neuropilar branches and axons of the giant interneurons in the firebrat seem to be homologous to those of crickets and cockroaches — they are strikingly similar in shape and position and the terminals of mechanosensory afferents occupy comparable regions of the neuropile. And neuronal patterns in embryonic crickets and adult firebrats can almost be superimposed, an example of ontogeny recapitulating phylogeny.

Dumont and Robertson's review[3] of some familiar neuronal circuits has a more didactic aim — to free their fellow circuit-breakers from the ideal that nervous systems are optimally designed. Their analysis suggests that years of searching have yielded few satisfactory examples of simplifying 'organizational principles' linking circuitry to behaviour. Nervous systems do not live by parsimony alone, it seems: simple patterns of behaviour are controlled by complex circuits and similar problems are solved in many different ways. In the search for understanding, the past has been ignored too much. Some features of neuronal circuits may have no functional significance but may be merely vestiges of an earlier pattern of behaviour. Among the examples the authors give are the serially repeated interneurons in the abdominal neuromere of the locust metathoracic ganglia that influence a thoracic pattern generator; and the presence in all abdominal ganglia of crayfish of synaptic connections that seem appropriate only to some. These anachronisms persist

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Erratum

IN the picture story on aphids (*Nature* **325**, 580; 1987) it is nepetalactone that gives catmint (*Nepeta cataria*) its characteristic smell, and not nepetalactol, as stated. The electron micrograph was taken by J. Hardie (Imperial College, Silwood Park).

Corrigendum

IN last week's leading article on the object 1987a "Supernova almost on the doorstep" (*Nature* **326**, 11; 1987) the words 'of this type' were inadvertently omitted from the sentence "Intriguingly, nobody has yet convincingly shown theoretically that supernovae of this type should occur at all".

because the animal has, so to speak, designed its way around them — old neurons have been incorporated into new patterns of behaviour or inappropriate connections have been overridden, though not necessarily replaced, by more appropriate ones.

Selection pressure acts on behaviour, not on neural circuits; as long as a circuit does its job, natural selection is powerless to pare it down. The difficulty, of course, comes in judging what has functional significance and what does not, given the limitations in our understanding of neuronal circuits. The evolutionary perspective provided by Dumont and Robertson[3] emphasises the value of comparative studies, such as those of Shaw and Meinertzhagen[1] and Edwards and Reddy[2], for providing the context in which these kinds of judgements can be made. ☐

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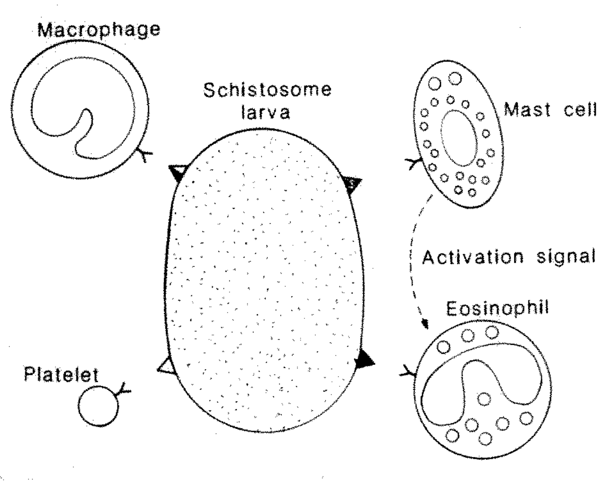
Parasitology

Cloned schistosome antigens

F.E.G. Cox

BECAUSE naturally acquired immunity to schistosomiasis is slow to develop and relatively ineffective, those hoping for a vaccine against this disease, which affects about 250 million people and countless cattle and buffalo, are looking at the possibilities provided by the techniques of gene cloning. After several false starts, this approach is now beginning to reap dividends. Professor André Capron and colleagues at Lille, France, in collaboration with a group at Transgène, report on page 149 of this issue[1] the isolation and expression of a gene, from adult *Schistosoma mansoni* worms, encoding an antigen that induces cytotoxic anti-parasite responses *in vitro* and protects a range of laboratory animals against schistosome challenge.



Immune responses in schistosomiasis are directed against the larval stage and involve macrophages, eosinophils, mast cells and platelets. Each cell type binds to a particular immunoglobulin which in turn recognizes a specific surface antigen. This results in the adherence of the cell to the schistosome larva and its subsequent destruction by antibody-dependent cell-mediated cytotoxicity.

Schistosome infections begin when the larval worm bores through the skin and makes its way to blood vessels where it matures. While in the circulation, the worm acquires a variety of host antigens that effectively render it immunologically invisible, and there is therefore no protective response to the adult worm. But both larval and adult worms produce antigens that elicit immune responses to subsequently invading larvae, which are recognized and destroyed. This destruction is a complex process involving several kinds of immunoglobulin, including IgE, and several cell types, including eosinophils[2] (see figure). Vaccines must mimic this situation to be effective and it has been widely assumed that they would be based on larval antigens.

But the experiments described in this issue[1] take as their starting point an antigen of relative molecular mass 28,000 (28K) from adult worms[3] quite distinct from others with a similar molecular mass[4]. Capron and co-workers used RNA from adult worms to synthesize complementary DNA which, when injected into *Escherichia coli*, produces a 28K protein with the same electrophoretic mobility as the native protein. The recombinant protein contains 211 amino acids but for their experiments Capron and colleagues used a 172-amino-acid 25K fragment that comprises the immunologically essential component of the native protein. Rats and hamsters recognize this recombinant protein and respond by producing specific IgG and IgE antibodies which also recognize the native molecule. When tested *in vitro*, the IgE in conjunction with eosinophils is instrumental in killing schistosome larvae.

Capron and co-workers followed their success with *in vitro* studies with vaccination trials using the recombinant protein. They found that it protected both rats and hamsters against challenge with schistosome larvae, shown by lower worm burdens in vaccinated animals compared with controls. Their final series of experiments demonstrates that vaccinated baboons produce cytotoxic antibodies similar to those found in rats and hamsters, suggesting that the vaccine is also effective in these primates.

The design of these experiments is important because rats and hamsters differ in their susceptibility to schistosome infections and the use of both species ensures that the results obtained pinpoint a fundamental mechanism of immunity. Baboons are the experimental animals most like man, so the successes achieved in all three species is encouraging when considering a human vaccine. Another encouraging feature is that the antigen used occurs in at least three other species of schistosome: *S. haematobium* and *S. japonicum* that infect man; and *S. bovis* that infects cattle, suggesting that the specificity of the vaccine is not too narrow. Successful vaccination of cattle against *S. bovis* using inactivated larval stages has already been achieved[5] and it is to be hoped that the 25K protein will also be effective. It will certainly be easier to administer.

These results only point the way to a vaccine; much remains still to be done. Immunity to schistosomiasis is complex and at least four cell types have been implicated in larval killing (see figure). The experiments described here concentrate on the IgE–eosinophil mechanism and no role has yet been described for the IgG that is also elicited by the recombinant protein. But it is now possible to imagine a vaccine consisting of several antigens, each eliciting a specific immunoglobulin, which in turn might activate its appropriate cell type. Even if this kind of vaccine is not completely effective, it would probably reduce the worm burden in individuals and thus contribute to control measures. There are mathematical models[6] that should predict how efficient a vaccine against schistosomiasis would have to be for its use to be a worthwhile proposition. ☐

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Geophysics

A mechanism for reversals?

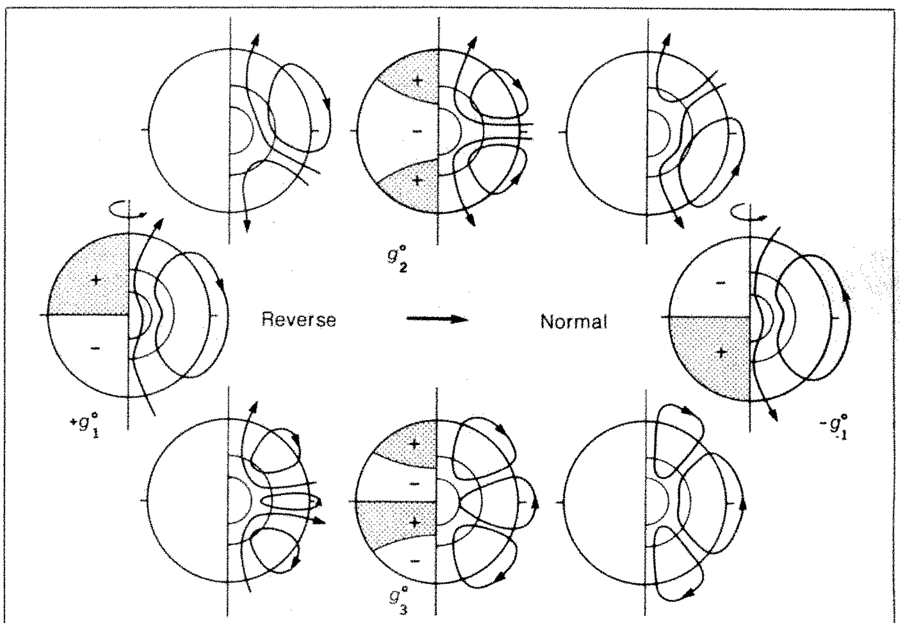
Mike Fuller

EVER since the demonstration of the reversal of the Earth's magnetic field, it has been hoped that this dramatic feature would provide a critical clue as to how the geodynamo operates. Another aspect of behaviour of the field studied with similar motivation is secular variation — the changes in the local geomagnetic field vector taking place over hundreds to thousands of years. Bloxham and Gubbins[1] have combined detailed studies of the recent field with a technique for projecting the surface observations downwards to produce a set of 'snapshots' of the field on the core-mantle boundary from 1715-1980. The approach is not quite as straightforward as the word snapshot suggests. Nevertheless, the work provides a new view of the geomagnetic field and has stimulated a reassessment of earlier ideas about secular variation. Now, on page 167 of this issue[2], Gubbins reports that the present decay of the dipole is caused by localized development of reversed flux in the Southern Hemisphere.

Gubbins relates this result to expulsion of toroidal flux by fluid upwelling, analogous to the processes in the Sun that give rise to sunspot formation and suggests that the growth and polewards migration of such reverse flux also provides a mechanism for reversals. His ideas are part of a new synthesis in geophysics, combining the efforts of seismologists and geomagnetists, which treats the Earth as a single system — a heat engine with events from the core to crust interrelated[3]. Thus, Gubbins suggests that the location of the fluid upwelling in the core is determined by the topography, or temperatures, at the base of the mantle, which is in turn controlled by mantle convection. These ideas have the virtue of being sufficiently specific to be testable against the palaeomagnetic record of reversals, as I will discuss.

Once the dust had settled on arguments about whether the geomagnetic field had actually reversed, or whether the palaeomagnetic record of reversals was caused by aberrant behaviour of rocks, two broad avenues of research opened up. First, attempts were led by Allan Cox to determine the history of reversal rates. As McFadden and Merrill note[4], there is now strong evidence for changes in the mean lengths of normal and reversed polarity intervals throughout geological time. Moreover, there is evidence that reversals are essentially a Poisson process. Thus reversals are a rare and random outcome of fluctuations in the the state of the field in the core and are independent of each other.

The second area of research to emerge was the description of what happens when the field is reversing. Such palaeomagnetic records of reversals are hard to come by because the geomagnetic field spends a very short time reversing, compared with time in the normal and reversed states. Nevertheless, records have now been obtained from lavas, intrusions and sedimentary rocks, and despite some discrepencies, a consistent picture of the major features is emerging.



Reversal of geomagnetic field via axial quadrupole (g_2^0) or octupole (g_3^0) transition field. The shading on the left-hand side of each sphere is the area of emergence or positive field, in a spherical harmonic representation. The right-hand side of each sphere shows magnetic field lines. With a + g_2^0 field all points on the surface of the Earth experience a 90° positive inclination sweeping from high southerly to high northerly latitudes. With a + g_3^0 field, the Southern Hemisphere sees a negative inclination of 90° sweeping from equator to pole, while a 90° positive inclination sweeps through the Northern Hemisphere. Although reversal transition fields are more complicated, reversals are likely to involve such field components. Gubbins's model seems to predict a quadrupole-like field with the Southern Hemisphere reversing first.

The field reverses polarity in a few thousand years with an associated reduction in field intensity to about 10 per cent of the value before the onset of the reversal. This reduction in intensity may last for about 10,000 years: the field may recover its former intensity briefly during reversals, but this is a controversial suggestion.

There have been attempts to establish the global morphology of the field during reversals, but multiple records of the same reversal are available only for the last few reversals, so that the problem is daunting. The records from the last reversal show that the field was not dominantly dipolar, so that some repartitioning of the energy among the different harmonics clearly took place. This is also shown by the increased dispersion of values of declination and inclination during all reversals.

At one point, it appeared[5,6] that the dominant spherical harmonic terms during the reversal might be low-order zonals. The dominance of these axisymmetrical harmonics explained how records of a reversal from sites at widely separated longitudes could all exhibit the observed high inclinations, as if the pole had passed beneath each site (see figure). Subsequent work points to the importance of non-zonal harmonics and to more complicated field morphologies.

There may yet be some worthwhile systematics to be gleaned from the records, despite the failure of the simple zonal models. Even though we know that the field is not dipolar during the reversal, the systematics are most conveniently established in terms of virtual geomagnetic poles (VGPs) — the pole of the dipole field that accounts for the observed declination and inclination at a site. Indeed, the most direct evidence that the field is not dipolar during reversals is that the VGP paths for the same reversal from different sites do not coincide. Clearly they would if the field were dipolar. Using the VGP construction, it turns out that the paths are frequently confined within a narrow band of longitude. (If westward drift were an important part of the non-dipole variation during a reversal, this would not happen.) We have not yet been clever enough to interpret this observation. There are also now several examples from North America, western Europe and the Soviet Union of sites at which different reversals have VGP paths confined to the same band of longitude. In one of the best-documented cases[7], three reversals with related paths took place over a period of about 1.4 million years and then there was an abrupt change.

It is now possible to test Gubbins's model[2] in the face of the available data. The time taken for the reversal is so short that models relying on the decay of the dipole field must be discounted. The model passes the test; it invokes the generation of negative flux, which eventually overcomes the initial field. It is also consistent with a relatively long period of low field intensity before and after the field polarity actually switches. While the negative flux is generated, the dipole field intensity should decrease without major increases in worldwide secular variation. Indeed, this is just what is happening at the moment. But what is seen is not necessarily the onset of a reversal — on a statistical basis the best bet is that the dipole field intensity will again recover, as it has frequently in the past, without reversing.

Gubbins's model also fits nicely into the Poisson framework of reversal epoch lengths. As he notes, the dipole field will often have experienced reduction in intensity through development of reverse flux, but only on rare occasions will the field actually reverse. Changes in reversal rates are easily accommodated by changes in mantle convection patterns on the appropriate timescale of 10^8 years.

The most critical test of the model comes from its prediction that all reversals should be similar within the lifetime of mantle convection patterns. Thus, the transition fields observed at particular sites should be similar. It is already known that reversals observed at the same locality are sometimes similar, but it is not clear whether they remain similar for the period required by the model. Preliminary indications are that the changes are too rapid to be consistent with it.

The constant location of the region of negative flux generation specified by Gubbins requires that reversals are all initiated in the same low-latitude region of the Southern Hemisphere. This is equivalent to a common starting point for a reversal model of the type suggested[8] by Hoffman, based on the Busse dynamo[9]. Gubbins and Bloxham note[10] that the core–mantle fields they calculate are similar to those predicted by Busse, although there are far more convection rolls in that model than predicted by the calculated fields. To the degree that Hoffman's model is successful in its predictions for the last reversal, Gubbins's model is also successful for that reversal, and it produces a mechanism to explain Hoffman's geometrical model.

Gubbins's model is not so obviously in conflict with available palaeomagnetic data on field reversals that it should be discounted. Unfortunately this, as is so often the case in the Earth sciences, gives a sufficient, but not necessary, condition. Some might argue that the model must be rejected because it is not consistent with the frozen-flux assumption. Gubbins, on the other hand, holds that the data show the frozen-flux assumption to be incorrect on the reversal timescale.

Whether the model turns out to be successful or not, it has already stimulated renewed interest in the reversal process. It is rooted in observations made possible by new techniques of analysis of the present and recent geomagnetic field and challenges palaeomagnetists to come up with the necessary data for a critical test. Such a test requires new emphasis on obtaining records of different reversals at individual sites, for which the best sources may well be ocean cores and rapidly deposited sedimentary sections. □

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Cell biology

The molecular basis for clathrin light-chain diversity

Dudy Bar-Zvi

COATED vesicles are involved in the transport of proteins between the organelles of eukaryotic cells and in receptor-mediated endocytosis, a specialized pathway by which cells take up specific molecules from the extracellular fluid. The coat of these vesicles has a characteristic polygonal structure of two protein complexes: assembly factor, composed of polypeptides of relative molecular mass 100,000–115,000 (100–115K) and 50K, and clathrin, composed of three 180K heavy chains and three 30–60K light chains, organized as a triskelion (refs 1,2; see cover picture). In two papers on pages 154 and 203 of this issue[3,4], Peter Parham and colleagues present data that help to elucidate clathrin light-chain structure and function.

There are several unexplained observations and unanswered questions about the function of clathrin light chains. Because the heavy chains alone can form triskelions that can self-assemble, it is not obvious that light chains are needed to generate a coated vesicle. They are required for the activity of the uncoating ATPase, a 70K heat-shock protein that catalyses the ATP-dependent removal of clathrin from coated vesicles *in vitro*[5]. Light chains can also stimulate the autophosphorylation of the 50K assembly factor polypeptide *in vitro*[6]. The significance of either of these activities *in vivo* is unknown.

With the possible exception of yeast, clathrin from other eukaryotic cells contains two distinct light chains[7] (called α and β, a and b, or sometimes 1 and 2). Moreover, clathrin light chains from brain are about 3K heavier than the corresponding light chains from other tissues[7]. What do these differences in mass signify? Because light chains from brain and from other tissues compete for binding to the same sites on the heavy chain near the centre of the triskelion[8,9]; because either the α- or the β-light chains of brain can satisfy the requirements of the uncoating ATPase[5]; and because both bind calmodulin and calcium, the significance of the differences in mass is unclear. Indeed, all the light chains have common features, for example, they are all heat-resistant polypeptides and unusually sensitive to protease[7].

Triskelions isolated from various tissues show variability in the molar ratio of the two light chains, ranging from 1 α-chain: 2 β-chains in brain to almost exclusively α-chain in reticulocytes. This variability, together with the observed difference in the mass of brain-specific light chains and those from other tissues, has prompted the suggestion that different specific light chains function in determining the specific properties or function of different types of coated vesicles. But because the polypeptide pattern and light-chain stoichiometry of endocytotic and exocytotic coated vesicles from liver seem to be identical[10], there is no clear indication of why multiple but apparently functionally interchangeable light chains have evolved in most organisms. The only functional difference between the α- and β-light chains known so far is the differential phosphorylation of the β-chain by a coated vesicle-associated casein kinase II (ref. 11).

Knowledge of the molecular and structural details of clathrin light chains should help to elucidate their role and distinguish the functions of the different light-chain types. Jackson *et al.* in this issue[3] report the cloning of clathrin α- and β-light chains from bovine brain and from lymphocytes. The α- and β-light chains are encoded by two closely related genes, so that the amino-acid sequences of the two light chains are 60 per cent identical. Comparison of the nucleic-acid sequence for both kinds of clathrin light chains shows that the brain light chains contain a hydrophobic insert of 30 and 18 amino acids for the α- and β-chains, respectively. The new results also show that the tissue specificity of these polypeptides results from different splicing events.

Brodsky *et al.* in this issue[4] map the different domains on the clathrin light chains using a panel of monoclonal antibodies. One region, residues 93–157, turns out to be inaccessible in native triskelions and coated vesicles, but accessible in free light chains. The authors therefore conclude that this region is involved in binding to the heavy chains. Another region, residues 158–208, is exposed in native triskelions and coated vesicles, and the authors suggest it is involved in coated-vesicle diversity. They also demonstrate that the brain-specific sequences are exposed in assembled clathrin, as expected.

Of great interest is the similarity in sequence of the carboxy-terminal clathrin light chains with the α-helical domains of various intermediate filament proteins[3]. Surprisingly, this homologous sequence, spanning about half the molecule, covers both the heavy-chain binding and the coated-vesicle diversity domains analysed by Brodsky *et al.*[4], raising questions about the evolution of light-chain specificity.

These cloned genes should make it possible to explore the functional difference between the α- and β-light chains by shifting their ratio either by overproduction of one light chain or by introduction of anti-sense RNA resulting in the underproduction of one light chain. Furthermore, knowledge of which domains on the light chains are responsible for light-chain–heavy-chain binding and which domains can account for light-chain specificity should facilitate experiments using site-directed mutagenesis to elucidate further the role of clathrin light chains, the significance of two different light chains in each cell, and the role of the brain-specific inserts. □

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Allan V. Cox (1926–1987)

ALLAN COX, Professor of Geophysics at Stanford University in California and principal architect of the magnetic polarity reversal timescale, one of the pillars of plate tectonics, died in an accident on 27 January. Shortly before his death he had announced his intention to return to teaching and research after nearly 8 years as Dean of Earth Sciences.

In the revolution of the Earth Sciences of the 1960s Cox, then at the US Geological Survey, played an essential role by determining and dating, in carefully chosen thick sequences of lava flows, the history of reversals of the Earth's magnetic field for the past several million years. With this work F. J. Vine and D. H. Matthews of the University of Cambridge were able to demonstrate that the oceanic crust of mid-ocean ridges faithfully records the magnetic reversals as it formed on ridge crests and was subsequently carried away laterally. Thus they confirmed the reality of the hypothesis of seafloor spreading, conceived a few years earlier by H. H. Hess at Princeton. Today the polarity-reversal timescale, extended now into the Mesozoic, has evolved to become a powerful chronostratigraphical tool of high resolving power and an essential part of the most recent geological timescales

During the 1950s, work on the palaeomagnetic properties of ancient rocks had shown, largely through the efforts of S. K. Runcorn, then at Cambridge, that wegenerian continental drift was to be taken seriously as a possible explanation for the observed multiple polar paths. In the ensuing discussion older observations, indicating that the Earth's magnetic field had episodically reversed its polarity, attracted little attention. Cox recognized the potential importance of this process, providing that the reversals could be shown to be synchronous, world-wide and not caused by some process within the rocks themselves. Together with Richard R. Doell he succeeded in persuading the Geological Survey of the value of systematic further study of this phenomenon and began working on the project in 1959, soon joined by Brent Dalrymple who furnished the essential isotope dating.

In a very short time the three, together with the Australian team of Ian McDougall and D. H. Tarling, produced the first and already remarkably sophisticated magnetic-reversal timescale, just in time for Vine and Matthews to use it in sending the geological revolution on its way to victory in 1968. In brief essays accompanying a compendium of key research papers from this period (*Plate Tectonics and Geomagnetic Reversals, 1973*) Cox commented perceptively on the human as well as the scientific events.

In recent years, working largely with his students because university and national science concerns made ever-increasing demands on his time, Cox turned to that other aspect of palaeomagnetism, the possibility of reading the original orientation and the latitude of formation of a rock from its magnetic inclination and declination. A life-long love for field work in the western United States did its part in determining his target, the geology of the collision zone between the North American and Pacific and associated minor plates. It turned out to be a superb choice because this work, now augmented by that of many others, has shown that our initial views of converging plate boundary processes were far too simple. The discovery that at convergence zones innumerable pieces of oceanic flotsam, 'exotic terranes' of often distant provenance, are aggregated and deformed, has returned to the mountain-building process that individuality of local character and history that plate tectonics in its first flush of pride had promised to erase.

Appointed in 1967 to the professoriate at Stanford, Cox was a teacher of great heart, capable of inspiring students well beyond the confines of geophysics. Quite often (and that may well be not as unique as our formal traditions pretend) he chose his subjects for research as much because of the people with whom he liked to work as by a coldly intellectual assessment of their potential.

Not surprisingly, he received many honours from his peers; among others the Vetlesen and Arthur Day medals, and memberships in the National Academy of Sciences, the American Academy of Arts and Sciences and the American Society of Philosophy.

As Dean of Stanford, treading carefully between its strong traditions of basic and applied Earth Sciences, he was instrumental in rejuvenating and invigorating the school. Balancing great respect for the judgement and advice of others with continuous insistence on high excellence and performance, he brought strength, youth and a forward look to its faculty and programmes.

Somewhat to the amazement of his friends, this shy and unassuming man also developed remarkable skill in fund-raising. Deeply humane although somewhat reserved as he was, these administrative activities, not always fully understood by his colleagues, must at times have been a heavy burden to him, first undertaken from a sense of duty but later, one hopes, valued for the sense of accomplishment they provided.

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Neutrinos from the recent LMC supernova

SIR—The supernova explosion that was observed recently[1] in the nearby Large Magellanic Cloud (LMC) provides a unique opportunity to test the theory of neutron star formation in Type II supernova explosions. In particular, one can investigate the prediction that almost all of the gravitational binding energy of the residue neutron star (~3×10^{53} erg) was radiated within a few seconds in the form of ~10^{58} neutrinos with average energy ~10–15 MeV. Here, we calculate the expected neutrino signals in terrestrial detectors which, according to the standard supernova theory[2–10], should have been observed during a short period (~1 s) before the onset of the optical supernova. (A measurement of the time delay between the first neutrino and optical pulses would be of great importance for our understanding of supernovae.) We also show how information on the fundamental properties of neutrinos (their life-times, masses and mixings, and their magnetic moments) can be obtained from neutrino observations of the LMC supernova. The number of neutrino-induced events in a terrestrial detector due to differential fluxes $d\phi/dE$ of neutrinos of types ν_i ($\nu_\phi=\nu_e$, $\bar{\nu}_e$, ν_μ, $\bar{\nu}_\mu$, ν_τ, $\bar{\nu}_\tau$) is given by

$$N=\Sigma_{ij}n_j \int \frac{d\phi_i}{dE}\,\sigma(j,\nu_i,E)dE \qquad (1)$$

where n_j is the number of particles of type j (atomic nuclei and electrons) in the target and $\sigma(j)$ are their cross-sections for relevant reactions which depend on the neutrino type and on the neutrino energy.

Recently Wilson[7] and Wilson *et al.*[10] have calculated the collapse of massive stars of 10, 15 and 25 $M_\odot$ and found that the predicted time-averaged neutrino signal does not vary greatly from star to star. In Table 1 we list the neutrino fluxes at the source as calculated by Wilson for a supernova explosion of a 25 $M_\odot$ star with a 1.6 $M_\odot$ iron core and the fluxes expected at a distance of 50 kpc from the supernova.

We have calculated the event rates in existing solar neutrino detectors using the spectra and fluxes of Wilson[7,10] and neutrino cross-sections from Bahcall (ref. 11 and unpublished data). Kamiokande II[12], which contains 3 kt of water, should experience about 50 events with a positron recoil energy in excess of 10 MeV. The events should all be concentrated within a few seconds at an epoch that was a short time before the onset of the optical supernova on 24 February 1987. The high time resolution of the detector and the bunching in time of the real events should make the signal stand out above noise by a large factor. The 140-t liquid-scintillator detectors of the University of Pennsylvania[13] in the Homestake mine and the 90-t scintillator detector in the Mont Blanc Tunnel[14] should have seen about 3 events each if the standard supernova theory is correct. This signal is marginally detectable because of the high time resolution of the experiments. A comparison of the arrival times for neutrino events seen in different detectors will provide a crucial test of the physical reality of the observations; the neutrinos should arrive essentially simultaneously at all terrestrial detectors. For the ^{37}Cl tank of Davis[15], only one additional ^{37}Ar atom is expected to be produced, a signal which would not be detectable.

Table 1 Neutrino intensities and temperatures

ν Type	$N_\nu(\times10^{57})$	T(MeV)	$\phi_\nu(\times10^{10})$
ν_e	4.5	4.7	1.6
$\bar{\nu}_e$	3.1	5.0	1.1
ν_μ	2.3	10.0	0.8
$\bar{\nu}_\mu$	2.3	10.0	0.8
ν_τ	2.3	10.0	0.8
$\bar{\nu}_\tau$	2.3	10.0	0.8

Number, N_ν, of neutrinos produced at the source and the flux (per cm^2), ϕ_ν, a distance of 50 kpc from a supernova in a 25 $M_\odot$ star with a 1.6 $M_\odot$ iron core[10]. Here T is the average temperature of the neutrinos in MeV.

Standard supernova theory may be wrong. It is more difficult to calculate how a star explodes than to estimate how the Sun shines and we do have the solar neutrino problem. To show the power of observations with existing neutrino detectors, we assume that 3×10^{53} ergs are emitted as thermal neutrinos (temperature T) and that the energy is distributed equally among all neutrino flavours. We find the following total number of events for the three different classes of neutrino detector mentioned above:

$$\text{events in 1 kt of } H_2O = 2.5\left(\frac{T}{1\,\text{MeV}}\right) \qquad (2a)$$

$$\begin{array}{l}\text{events in 0.1-kt-}\\ \text{liquid scintillator}\end{array} = 0.35\left(\frac{T}{1\,\text{MeV}}\right) \qquad (2b)$$

$$\begin{array}{l}\text{events in 0.6-kt}\\ {}^{37}\text{Cl detector}\end{array} = 0.007\left(\frac{T}{1\,\text{MeV}}\right)^{2.7} \qquad (2c)$$

(The last relation overestimates the detection rate for temperatures above 5 MeV; it is too large by a factor of about 2 at $T=10$ MeV). A thermal spectrum with a typical energy of T~10–15 MeV would produce an event rate for the ^{37}Cl experiment that is an order of magnitude larger than the standard supernova model.

A number of authors, motivated by the solar neutrino problem[16], have suggested recently that the neutrino has properties not included in the simplest electroweak model. Explanations of the solar neutrino problem of this type include neutrino decay[17], resonant neutrino oscillations[18] and a large neutrino magnetic moment (L.B. Okun *et al.*, preprint ITEP, 1986). The LMC supernova can be used to test these suggestions.

If the ~10 MeV ^{8}B solar neutrinos decay significantly during their flight from the Sun to Earth, then essentially no neutrinos will survive the flight from the LMC supernova at a distance of 50 kpc. Consequently, the neutrino decay hypothesis which may explain the solar neutrino problem predicts no neutrino signals in underground neutrino detectors from the recent LMC supernova. If neutrinos are detected, their life times in the laboratory frame must exceed 10^5 years.

Neutrino oscillations increase the expected signals because the average energy of the ν_μs and ν_τs is about twice that of the ν_es (see Table 1). Electron neutrinos generate most of the signals and the interaction cross-sections increase with incident neutrino energy. If the ν_μs and ν_τs oscillate into ν_es (and vice versa) after leaving the neutron star (for example, through the MSW[18] effect on positrons and electrons in the outer layers of the star) then the incident ν_es will have higher energies and higher cross-sections and consequently will have more events in terrestrial detectors. Complete interchange between ν_es and ν_τs or ν_μs will increase the signals in water and liquid scintillators by about a factor of two but will still produce only ~1 event in Davis's solar neutrino experiment.

Dar[19] has shown that if the neutrino magnetic moment is in the range 10^{-10} to 10^{-12} Bohr magnetons (which explains the solar neutrino problem (L.B. Okun *et al.* preprint ITEP, 1986)), then relatively-high-energy neutrinos with a thermal spectrum can escape from a supernova explosion as a result of magnetic interactions with electrons. A conservative assumption, within this non-standard picture, is that the ν_e neutrinos are emitted with a temperature in excess of 10 MeV (and with about half the fluxes assumed for the thermal case cited above) (see ref. 19). The event rates for this model can be obtained from equation (2) by substituting the relevant temperature and dividing by two (to correct for smaller fluxes).

The collapse event produces a fluence of ~$3\times10^{58}\,(m_i/T)^{3/2}e^{-m_i/T}$ of neutrinos of flavours i with mass m_i. This flux contains a significant amount of energy if the neutrino mass, m_i, is less than a few hundred MeV. If these massive neutrinos are unstable and decay electromagnetically (see refs 20–22), for example, via $\nu_H\rightarrow\nu_e+e^+ +e^-$ or other electromagnetic decay modes, ~$10^{53}f(m_i/T)^{3/2}e^{-m_i/T}$ ergs will be produced in electron–positron pairs and γ-rays between the Earth and the supernova ($f=1$ if the neutrino life time, τ, is shorter than 5×10^{12}s, the flight time from the supernova to the Earth, and $f=\tau/5\times10^{12}$ otherwise). In order to observe an electromagnetic signal from this decay, it must take place outside the collapsing star

($\tau \geqslant 10^3$ s). If more than $10^{-12}/[f(m_i/T)^{3/2} e^{-m_i/T}]$ of this energy is converted to γ-rays, within one second (either by direct decay or by rapid electron–positron annihilation), we should detect a γ-ray burst coincident with the ν-signal. The absence of such a burst coincident with the ν-signal can be used to eliminate electromagnetic decay modes of neutrinos with masses in the range of one to a few hundred MeV and lifetimes larger than ~10^3s.

Higher-energy neutrinos will arrive at the Earth before lower-energy neutrinos if the neutrino has a finite mass. However, this time delay, ~0.01 $(m_\nu/1 \text{ eV})^2(10 \text{ MeV}/E_\nu)^2$ s, is too small to be observable for the LMC supernova.

A yet unknown, but potentially significant, fraction of the released energy might be emitted as gravitational radiation. The gravitational wave emission depends strongly on the asymmetry of the collapse and in particular on the amount of rotation of the core. Even with the most optimistic assumptions about gravitational radiation emission, $E_{gr}=0.01\ M_{core} \approx 0.016\ M_\odot$, and $h=\Delta l/l \approx 0.7 \times 10^{-18}$, the LMC supernova is just below the sensitivity of current detectors. However, it is interesting to ask whether gravitational radiation would have been seen by next generation detectors (such as the proposed Caltech/MIT interferometer) with a sensitivity of 10^{-21}. At low angular momentum, the gravitiational radiation emissivity scales[23] like J^4. Therefore, the LMC supernova would have been detected even if the core was rotating only at 1/30 of its breakup speed, which is as slow as the Crab pulsar today.

Note added in proof: Since this paper was received on 2 March the neutrino burst was found by the Kamiokande experimental group, with properties generally consistent with the calculated expectations.

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Sulphated polysaccharide structures

SIR—Anyone with a knowledge of the actual structures of the molecules will have been amused by the debate between Lubec and Cole *et al.* on the validity of the use of heparin as a functional analogue of heparan sulphate in N-CAM-mediated cell adhesion studies, and the use of chondroitin sulphate as a control molecule in polysaccharide binding studies[1].

In their response, Cole *et al.* circumvent much of the structural argument but they do point out that, contrary to Lubec's claim, heparin does contain *O*-sulphate groups and so chondroitin sulphate is not such a bad control. Their case might have carried greater conviction had they not just mentioned the 3-*O*-sulphates but also pointed out that heparin actually contains a higher concentration of *O*-sulphates than chondroitin sulphate though these are present mostly as 6-*O*-sulphates and 2-*O*-sulphates. The 3-*O*-sulphate groups occur only rarely and in a minority of the heparin chains.

As to heparin and heparan sulphate, both are negatively-charged and composed of an alternating arrangement of α, β-linked glucosamines (GlcN) and hexuronate. The GlcN units may be *N*-sulphated or *N*-acetylated. In heparin over 80 per cent of the GlcN units are *N*-sulphated and in total there are over two sulphate groups per disaccharide. In heparan sulphate there are approximately equal numbers of *N*-sulphated and *N*-acetylated glucosamine residues arranged in a predominantly segregated fashion. The *O*-sulphates are always in very close proximity to the *N*-sulphates, forming sulphate-rich domains which co-exist with non-sulphated acetyl-rich regions in the intact polymer. The sulphate-rich areas are heparin-like, though they rarely possess the sulphate density found in heparin[2,3]. The key point is that the sulphate clusters are spaced-out along the polysaccharide chain. Heparin cannot reproduce this lateral asymmetry in polymer sulphation which is probably very important for the functional specificity of heparan sulphate. Nevertheless, because the sulphate groups in heparin are of a similar type to those in heparan sulphate, it is the best available functional analogue. It must also be stressed that heparan sulphate is not a single entity. The content of *O*-sulphate groups is variable and shows some correlation with cell lineage and cell proliferation[4]. These variations are probably essential for the cell-type specific functions of the polymer.

Finally , Lubec is wrong to imply that heparan sulphates ensnare enormous numbers of proteins in an ionic embrace. They bind selectively to different types of collagens, they do not all bind to fibronectin and only a small proportion bind to anti-thrombin III. They are much too fastidious in their interactions to be referred to as molecules with 'glue-activity'.

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Alzheimer's disease and aluminium

SIR—At a time when an article in *Nature* can send securities plunging in exchanges all over the world (in this case the aluminium manufacturing companies' stocks), the opening paragraph of K. Tennakone and S. Wickramanyake's letter [1] is not nearly as cautious as it should have been.

The aluminium model for Alzheimer neurofibrillary tangles died[2] in the same journal issue it was born[3] and kept a semblance of after-life for grantmanship reasons only. High levels of aluminium in senile plaques are not indicative of a causative role of the metal: the neurotoxic effects of aluminium bear no pathological resemblance to Alzheimer's disease, as has been shown in numerous studies of dialysis encephalopathy. And, studies of large kindreds with Alzheimer's disease[4] show no environmental influence whatsoever on the incidence of the disease.

Persistent misunderstanding about the putative role of aluminium in Alzheimer's disease prompted me recently to publish a report of an old case of accidental metallic aluminium implantation in the human brain[5]: the ensuing clinico-pathological picture of intractable epilepsy with diffuse gliosis, without any lesion reminiscent of Alzheimer's disease, ought to put the matter to rest.

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Transmission dynamics of HIV infection

Robert M. May and Roy M. Anderson

Simple mathematical models of the transmission dynamics of human immunodeficiency virus help to clarify some of the essential relations between epidemiological factors, such as distributed incubation periods and heterogeneity in sexual activity, and the overall pattern of the AIDS epidemic. They also help to identify what kinds of epidemiological data are needed to make predictions of future trends.

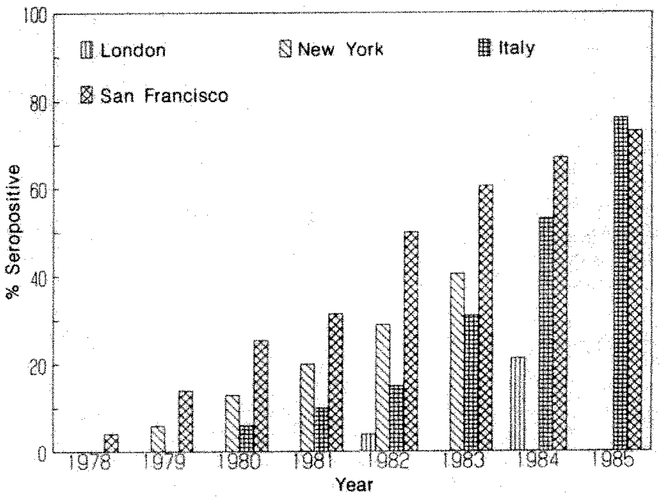

Fig. 1 The rise in seropositivity to HIV antigens in cohorts of patients over the period 1978–1985. The studies in San Francisco[12], London[23] and New York[35] were of homosexual/bisexual males. The study in Italy[24] is of drug addicts.

DESPITE remarkable advances in understanding the basic biology of human immunodeficiency virus (HIV — the aetiological agent of AIDS, acquired immune deficiency syndrome)[1-5] public health planning continues to be hampered by uncertainties about epidemiological parameters[4-6]. Accurate information about the typical duration and intensity of infectiousness, or about the fraction of those infected who will go on to develop AIDS (and after how long), will emerge only from carefully designed studies on these same timescales, which is to say many years. In the absence of such information, mathematical models of the transmission dynamics of HIV cannot be used at present to make accurate predictions of future trends in the incidence of AIDS, but they can facilitate the indirect assessment of certain epidemiological parameters, clarify what data is required to predict future trends, make predictions under various specified assumptions about the course of infection in individuals and patterns of sexual activity within defined populations (or changes therein) and, more generally, provide a template to guide the interpretation of observed trends[7,8].

Whether an infection can establish itself and spread within a population is determined by the key parameter R_0, the basic reproductive rate of the infection[7]. R_0 is the average number of secondary infections produced by one infected individual in the early stages of an epidemic (when essentially all contacts are susceptible); clearly the infection can maintain itself within the population only if R_0 exceeds unity[9,10]. For a sexually transmitted disease (STD), R_0 depends on c, which is essentially the average rate at which new sexual partners are acquired, on β, the average probability that infection is transmitted from an infected individual to a susceptible partner (per partner contact) and on D, the average duration of infectiousness[7,11]. In what follows, we mainly restrict attention to the spread of HIV among homosexual males, now responsible for the bulk of AIDS cases (about 70–80% in the United States, and a similar proportion in European countries[6,12,13]).

Initial stages of the epidemic

The characteristics of most STDs cause their epidemiology to differ from that of common childhood viral infections[11,14,15]. Unlike infections caused by airborne transmission, the rate at which new infections are produced is not dependent on the density of the host. Second, the carrier phenomenon in which certain individuals harbour asymptomatic infection is often important (as in the spread of herpesvirus). Third, many STDs induce little or no acquired immunity on recovery (for example, gonorrhoea) and fourth, net transmission depends on the degree of heterogeneity in sexual activity prevailing in the population.

As Hethcote and Yorke[11] have shown in their studies of gonorrhoea, mathematical models for the dynamics of STDs need to take account of the substantial variations of sexual activity within the population at risk. A particular risk group (such as male homosexuals in San Francisco[12]) of total size N may be roughly partitioned into subgroups of size N_i, each of whom on the average acquire i new sexual partners per unit time (when $N = \sum_i N_i$). The probability that susceptible individuals in this ith group will become infected, per unit time, is thus $i\lambda$, where λ is the probability that infection is acquired from any one new partner. In turn, λ is equal to the product of the transmission probability β defined above and the probability that any one randomly-chosen partner is infected (with such partners being more likely to come from the sub-groups of individuals with high degrees of sexual activity).

Exponential growth

When these assumptions are incorporated into a model for the transmission dynamics of HIV infection, the infected fraction of the population at risk (who are seropositive in tests for HIV) rises exponentially, as $\exp(\Lambda t)$, in the early stages of the epidemic. The exponential growth rate, Λ, is related to the basic epidemiological quantities defined above by:

$$\Lambda = \beta c - 1/D \qquad (1)$$

The effective average over the distribution by degrees of sexual activity, c, is given explicitly as

$$c = \Sigma i^2 N_i / \Sigma_i N_i = m + \sigma^2/m \qquad (2)$$

where m is the mean and σ^2 the variance of the distribution of the number of new sexual partners per unit of time[8]. Thus, c is not simply the mean but the mean plus the ratio of variance to mean, which reflects the disproportionate role played by highly active individuals (in the tail of the probability distribution of sexual activity), who are both more likely to acquire infection and more likely to transmit it. The basic reproductive rate for HIV infection, R_0, is related to the parameters β, c and D, and hence to Λ by the formula

$$R_0 = \beta c D \qquad (3)$$

In contrast with standard epidemiological models in homogeneous populations (where the exponential phase of rising incidence lasts until something like half the pool of susceptibles have been infected), the early exponential phase is of relatively short duration in our HIV models, giving way to a more nearly linear rise in the fraction infected (see Fig. 2).

This is because most susceptibles in the sexually highly active categories are infected in the early stages of the epidemic, producing saturation effects in these categories which decrease the exponential rise in incidence within them; although the incidence of infection continues to rise among individuals in less sexually active categories, the overall rate of increase is now slower than exponential.

Much less information is available about the rise in the number of individuals infected with HIV, as a function of time, than about the rise in the subsequent incidence of AIDS[46,12,13], largely because information about infection requires serological examination for antibodies to the HIV virus. Although the initial infection may produce symptoms[3,16,17] and, in some cases acute encephalopathy[18] and meningitis[16], it is not clear that such symptoms are always evoked: in any event, the symptoms are usually sufficiently mild to preclude systematic reporting. By contrast, the opportunistic infections cancers[4,5,12,19], and subsequent mortality characteristic of the destruction of the immune system in AIDS, leads to fairly reliable reporting[20]. There is, however, one study of hepatitis B virus (HBV) in a cohort of 6,875 homosexual and bisexual males in San Francisco, which resulted in serum samples being taken and preserved as early as 1978[12,2122]; stored sera of a representative sample of 785 of these individuals gives the rise in the fraction seropositive for HIV, from 1978 to 1985, shown in Fig. 1.

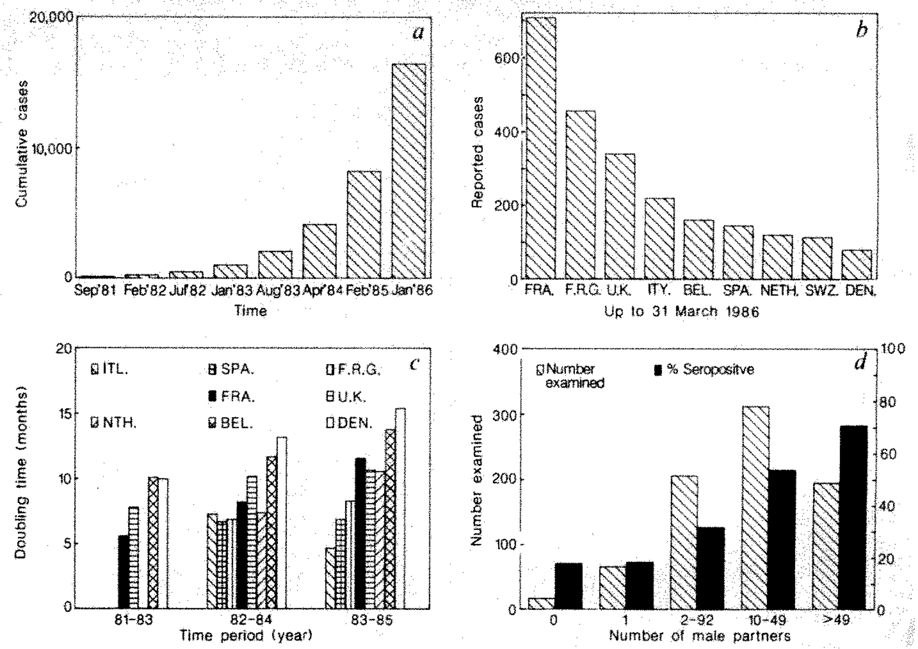

Fig. 2 *a*, The rise in the cumulative number of reported cases of AIDS in the USA over the interval September 1981 – January 1986[13]. *b*, Reported cases of AIDS in 9 countries of the European Community up to 31 March 1986[40]. *c*, Doubling times in the cumulative incidence of AIDS (t_d) recorded in months for various European countries[40] over various time intervals (1981–83, 1982–84, 1983–85; DEN, Denmark; BEL, Belgium; NTH, Netherlands; FRA, France; E.C., European Community in total; F.R.G.. Federal Republic of Germany; SPA, Spain; ITL, Italy; UK, United Kingdom). *d*, The relationship between sexual activity amongst a sample of homosexual/bisexual males (from San Francisco, USA) as measured by the number of male partners over a two-year period, and the percentage of each group (based on sex partners) who were seropositive for HIV antibodies (data from ref. 26).

Table 1 Doubling time of the HIV epidemic (in the early stages)

Area	Period	Doubling time t_d (in months)
Serological data		
(a) Male homosexuals		
San Francisco, USA	1978 – 80	10 – 11
New York City, USA	1979 – 80	10 – 11
London, UK	1982 – 84	9 – 10
(b) Intravenous drug users		
Italy	1980 – 83	15 – 16
London, UK	1983 – 85	11 – 12
Switzerland	1983 – 84	8 – 9
Case notifications		
(a) All risk groups		
Australia	1982 – 85	4 – 5
Austria	1983 – 85	15 – 16
Belgium	1982 – 84	11 – 12
Canada	1981 – 85	9 – 10
Denmark	1982 – 84	13 – 14
Europe (EC)	1982 – 84	8 – 9
France	1982 – 84	8 – 9
Italy	1982 – 84	7 – 8
Netherlands	1982 – 84	7 – 8
Spain	1982 – 84	6 – 7
Sweden	1983 – 85	8 – 9
Switzerland	1983 – 85	9 – 10
United Kingdom	1982 – 84	10 – 11
United States	1982 – 83	5 – 6
West Germany	1982 – 84	6 – 7
(b) Heterosexuals		
United States	1982 – 84	9 – 10
Average		9 – 10

The pattern of roughly linear rise shown in Fig. 1 is uncharacteristic of standard epidemics (in homogeneously mixed populations), but is suggested by our HIV models. In Britain and other countries in Europe, the virus seems first to have appeared several years later than in the United States (Fig. 2*a*, *b* and *c*), and the spread of infection is still in its early stages. As a result there are serological studies focused on HIV roughly from its initial appearance in Europe[23,24] (Fig. 1). The initially exponential rise in HIV infection may be characterized by a doubling time, t_d, related to the growth rate, Λ, of (1) by $t_d = (\ln 2)/\Lambda$.

Table 1 summarizes information about doubling times deduced from serological and case notification studies, which lead to a surprisingly consistent estimate of t_d ~8–10 months in the early stages of the epidemic (Fig. 2*d*) giving an estimate of Λ of about 1.0 yr^{-1}. The characteristic duration of infection (and infectiousness), *D*, is probably not significantly less than the characteristic time from HIV infection to manifestation of AIDS.

But *D* may be significantly longer if a substantial proportion of infected individuals remain asymptomatic carriers (with the epidemiology similar to hepatitis B virus[25]). On the other hand, recent studies observing that measurable HIV antigen (HIV-Ag, the presence of which indicates the presence of the virus) appears early and transiently in primary HIV infection, that antibody production follows (1–3 months after infection) and that HIV-Ag may then disappear could imply lower estimates of *D*, as would the apparent correlation of this persistence or reappearance of antigen with clinical, immunological and neurological deterioration[3].

In the absence of conclusive data on infectiousness during the incubation period, we shall assume that *D* is equal to the incubation period. Studies of cases of AIDS associated with transfusion suggest that the average incubation period is 4–5 years[31], but as such studies are extended, this estimate will rise (Fig. 3). The true average may be 8–10 years or more. Our estimate of Λ in conjunction with equation 1 then leads to the rough estimate

$$\beta c \simeq 1\,\text{yr}^{-1} \qquad (4)$$

Note that $\Lambda \simeq \beta c$ provided *D* is large (4–5 years plus). Thus data on changes in seropositivity over time have allowed us to infer the approximate magnitude of the combination of epidemiological parameters β and *c*, neither of which can easily be estimated directly.

Is this estimate consistent with what is known about β and *c* separately? Unfortunately, nearly all the information about degrees of sexual activity among male homosexuals has focused on average numbers of sexual partners, as distinct

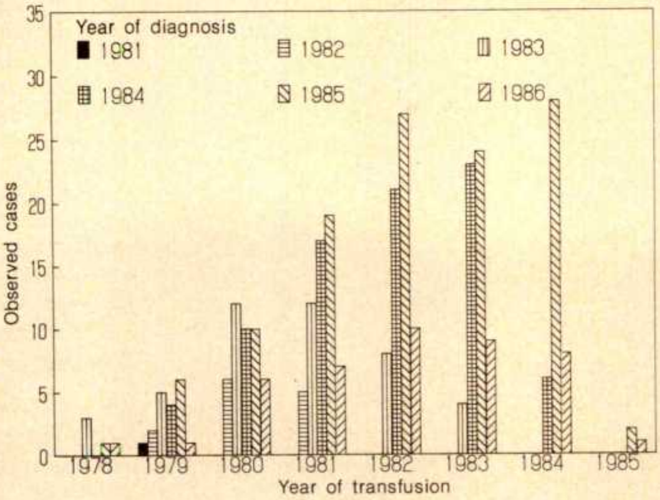

Fig. 3 Data on the distribution of the incubation period of AIDS derived from longitudinal studies of transfusion recipients (data from ref. 31). Observed cases of AIDS are recorded as a function of the year of transfusion (the assumed point of acquisition of infection) and the year of diagnosis. A Weibull distribution provides a good empirical description of this data with a mean incubation period of ~ 4–5 years.

from average number of new partners per unit time[26,27] (Fig. 4). For less active individuals (say, 1–3 partners per 6-month interval), the rate of acquisition of new partners will be seriously overestimated by the average number of partners. On the other hand, the quantity c is disproportionately influenced by highly active individuals, most of whose partners are likely to be new, so that studies based simply on numbers of partners may give a rough guide to the magnitude of c (Fig. 4). Quantitative information on average values of β, whether for homosexuals or heterosexuals, is very limited at present. Estimates vary widely (from 0.05 to 0.5) although it appears that the average probability of transmission per partner contact is higher among male homosexuals than among heterosexuals, perhaps as a result of more frequent sexual activity that results in epithelial damage (for example anal intercourse)[26,29]. Our estimates of $\beta c \sim 1\text{yr}^{-1}$ together with the high estimates of c for homosexuals suggest that β may be small (~ 0.05). But estimates of c based on the reported number of partners per unit of time may significantly overestimate the number of new partners per unit of time, which, or that equating D to the incubation period, may overestimate the average duration of infectiousness. It may also be that the high values of c arise from sampling biased towards the high activity groups of homosexual communities.

As public awareness about AIDS has increased, there have been changes in patterns of sexual activity among male homosexuals in the United States (reflected, for example, in marked decreases in the incidence of rectal gonorrhoea[29,30]) which have presumably resulted in changes both in β and in c[27]. Our discussion, therefore, pertains mainly to the relatively early stages — 1978 to the early 1980s — of the epidemic.

Incubation period

Although much more information is available about the incidence of AIDS than of HIV infection (Table 1), it is harder to tease estimates of epidemiological parameters out of these. Incidence of AIDS depends not only on the transmission factors β and c, but also on the incubation period and on the fraction, f, of those infected who will eventually develop AIDS.

Significantly, estimates of both the incubation period and f have tended systematically to increase since the epidemic was first recognized[4,5,28,31,32]. Estimates of f range grom 10% to 75% or more[19,28,33,37], with an incubation period of 4–5 years or more[31]. The progressive sequence of steps which eventually impair the ability of the immume system to respond to opportunistic infection seem not to be reversible. But whether all those infected with HIV are moving toward AIDS at different rates, or whether some will develop AIDS while others never will, remains unclear. Variability in the incubation period, and whether or not an infected person develops AIDS, could be accounted for by genetic heterogeneity within the host population (HLA-linked[38,39]), or could be associated with specific strains of the antigenically variable HIV virus[1,6].

Studies of the incubation period for those who develop AIDS suggest that the 'hazard function', the probability of the disease manifesting itself as a function of the time since infection, increases with time (Fig. 3). Lui and co-workers[31] have assumed a Weibull distribution (a flexible two parameter probability distribution) for the incubation period with probability density function

$$h(t) = \gamma v^{-\gamma} t \exp[-(t/\gamma)^{\gamma}] \qquad (5)$$

If indeed the probability per unit time, to develop AIDS (for that fraction f who do indeed develop it) increases linearly with time from infection as αt, the result is a Weibull distribution with $\gamma = 2$ and $v = \alpha$ for the hazard function[8]. This

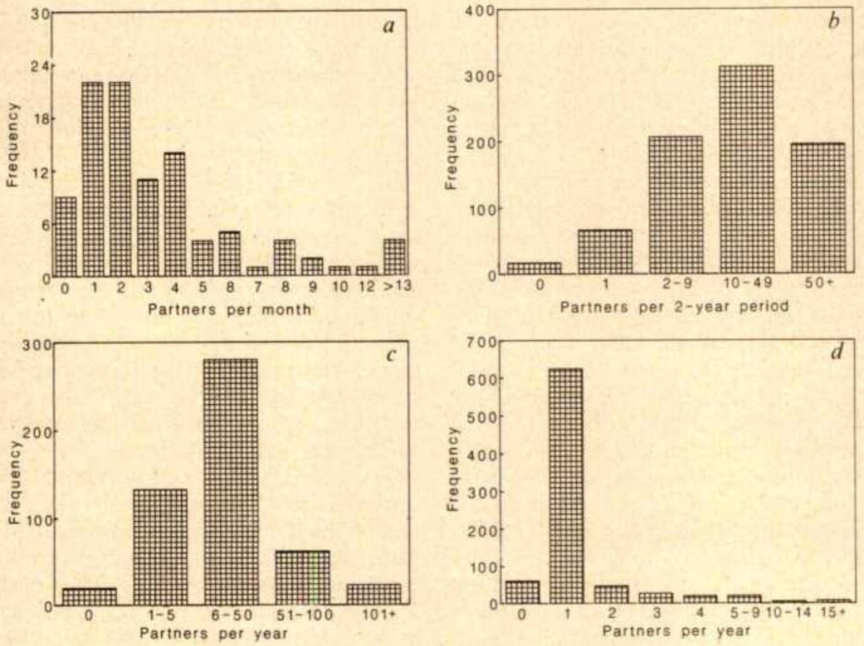

Fig. 4 Studies of sexual activity amongst $a-c$, male homosexual and d heterosexual communities. The graphs record frequency distributions of the number of sex partners per defined time period in samples of homosexual/bisexual males and heterosexuals. a, Homosexual/bisexual males resident in London surveyed in 1986 (unpublished data from C.A. Carne and I.V. Weller) (m = 4.7 per month, $\sigma^2 = 56.7$). Data denote male partners per month. b, Homosexual/bisexual males resident in San Francisco surveyed in 1984–85 (data from ref 26). Data denote male partners per 2-year period. c, Homosexual/bisexual males resident in London surveyed in 1984 (unpublished data from T. McManus). Data denote male partners per year. d, Heterosexuals between the ages of 18–44 years in England surveyed in November 1986 (unpublished data, Harris Research Organisation; R.M.A. and G.F. Medley). Data denote partners of the opposite sex per 1 year period (sample size = 823, $m = 1.41$, $\sigma^2 = 4.36$). A further survey of homosexual men (ref. 27) in San Francisco reveals a decline in the mean partners per month over the period November 1982 to November 1984 from 5.9 to 2.5.

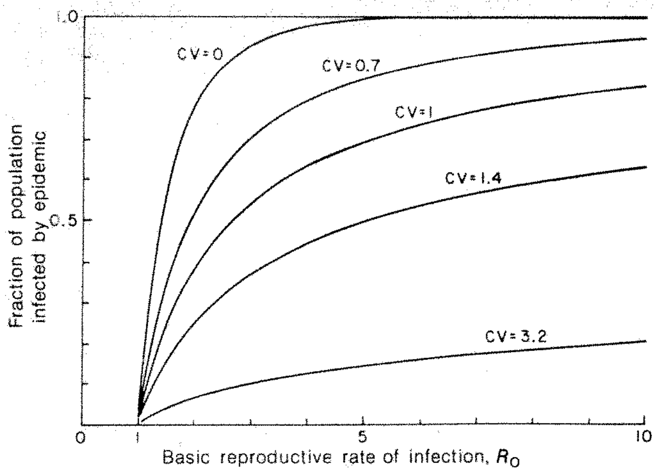

Fig. 5 The relationship between the eventual fraction infected (seropositive) in an epidemic of HIV and the basic reproductive rate R_0 (see text). The predictions are based on a model which assumes that sexual activity (defined as the number of new partners per unit of time) obeys a gamma distribution with varying coefficients of variation (CV) (mean m and variance σ^2; see text).

assumption differs from conventional epidemiological models, where infected individuals move through the incubation interval either at a fixed rate, or in a fixed time. But none of this resolves the question of what proportion of those infected will develop AIDS on what timescale: That issue will be resolved only by very long term (many decades) studies.

Fraction eventually infected

In a closed and homogeneously mixed population, the total fraction eventually infected depends only on the basic reproductive rate of the infection, R_0, defined above as shown[7] by the uppermost curve in Fig. 5. For sexually-transmitted infections such as HIV, the result can be extended to include the complications associated with a wide diversity in degrees of sexual activity.

In a closed population, the eventual fraction seropositive will depend both on R_0 and on the actual distribution of rates of acquisition of sexual partners. Assuming a gamma distribution[8], we may characterize it by c and by its coefficient of variation (CV = σ/m). The resulting overall fraction infected is shown as a function of R_0, for a range of values of CV, in Fig. 5; for fixed R_0, the eventual seropositive fraction can be much lower than for CV = 0, if the variability in degrees of sexual activity (measured by CV) is high. This makes intuitive sense: the highly active individuals acquire infection, and eventually are removed, relatively early in the epidemic; transmission among the remaining, less active, individuals may be relatively weak.

Figure 5 may be used, in combination with two factual observations, to make a rough assessment of R_0 for HIV among male homosexuals. First, the studies indicate great variability in degrees of sexual activity among male homosexuals (with CV significantly in excess of unity[8,26,27]), thus confining attention to the lower curves. The second observation is that levels of seropositivity to HIV among male homosexuals in San Francisco in 1985 are variously reported as 70% or more[12] (in the HBV study which is probably biased towards more active individuals) and as around 50% (in a study carefully constructed to avoid bias[26]), providing a lower bound of 50–70% on the proportion ever seropositive. For CV noticeably in excess of unity, this can be achieved only if R_0 is in excess of 5.

Thus our early estimate of $\beta c \sim 1\ \text{yr}^{-1}$, in conjunction with the assessment that R_0 exceeds 5, leads to an indirect estimate that D exceeds 5 years. Although R_0, like β and c changes with changing social and sexual habits, the data leading to our earlier estimate for βc come from the early stages of the rise in HIV infection, before such changes were significant. The estimate of R_0 depends importantly on observed levels of seropositivity, but these were also high before social changes became pronounced. Consequently, our estimate of D which depends only on the basic biology of HIV, is reasonably consistent. This independent estimate of $D \sim 5$ years accords with current estimates that the incubation period is 4–5 years or more.

An estimate of the value of R_0 in the early stages of the epidemic is also valuable in indicating the magnitude of the social changes needed to bring R_0 below unity. If R_0 is around 5–10 or more, then reductions by a factor of 5–10 or more in βc are needed. Because c depends disproportionately on those in the highly sexually active category, programmes aimed at getting them to change their habits — both to fewer partners and to "safe sex" — are most efficient. But if such individuals are less likely to respond to public health education, it will be harder to bring R_0 below unity.

Mortality

The frequent assumption that the severity of the epidemic, in terms of cumulative mortality, will be greatest if all those infected eventually develop AIDS and subsequently die is not necessarily true. Mortality depends critically on the duration of infectiousness of both those infected who develop AIDS and those infected who do not. If the latter have a similar life expectancy to those not infected, but remain infectious for life, they may contribute more to the net transmission of the virus, R_0, than those who die of AIDS. Much may be understood by recognizing that the overall net reproductive rate of the virus, R_0, is made up of two components, the reproductive rate of those who develop AIDS (R_{01}) and the equivalent rate of those who do not (R_{02}). If a fraction f develop AIDS

$$R_0 = fR_{01} + (1-f)R_{02} \tag{6}$$

where the two reproductive rates are defined by equation (3) with different parameters for the separate groups. Even if the asymptomatic carriers are less infectious than those who develop AIDS, if they remain infectious over, say, a 30-year span of sexual activity, R_{02} may be much larger than R_{01}, and, depending on f, the contribution of the asymptomatic carriers to R_0 may be dominant.

At present, it is not possible to tell whether the severity of the epidemic will be increased or decreased if a larger fraction of those infected develop AIDS, for the relative infectiousness of the two categories is unknown. For public health planning it is clearly important to attempt to acquire such data.

Dynamics of the epidemic

The dynamics of an HIV epidemic within a homosexual community are represented by the results of our calculations given in Fig. 6, which shows the proportion seropositive and the incidence of cases of AIDS as a function of time since the start of the epidemic. It is assumed that 30% of those infected eventually manifest AIDS, with the incubation intervals obeying a Wiebull distribution such that the average incubation period is 5 years[31]. Individuals who are incubating AIDS are assumed infectious throughout the incubation interval, and the 70% who remain asymptomatic are assumed to remain infectious for similar periods.

Many of the features presented in Fig. 6 show qualitative agreement with observation. The rise in incidence of infection (seropositivity) is initially exponential, but soon shows a more linear rise. And, the rise in incidence of AIDS lags that in the proportion infected, as seen.

It is easy to build epidemiological models of arbitrary complexity, which may appear beguilingly realistic, but we think there is little point in constructing them until more is known about the relevant epidemiological parameters. We distrust predictions made by using statistical procedures to fit polynomial or exponential curves to existing data on the incidence of AIDS, and then extrapolating[40,44]. The HIV epidemic is a dynamic process; to predict future trends, models

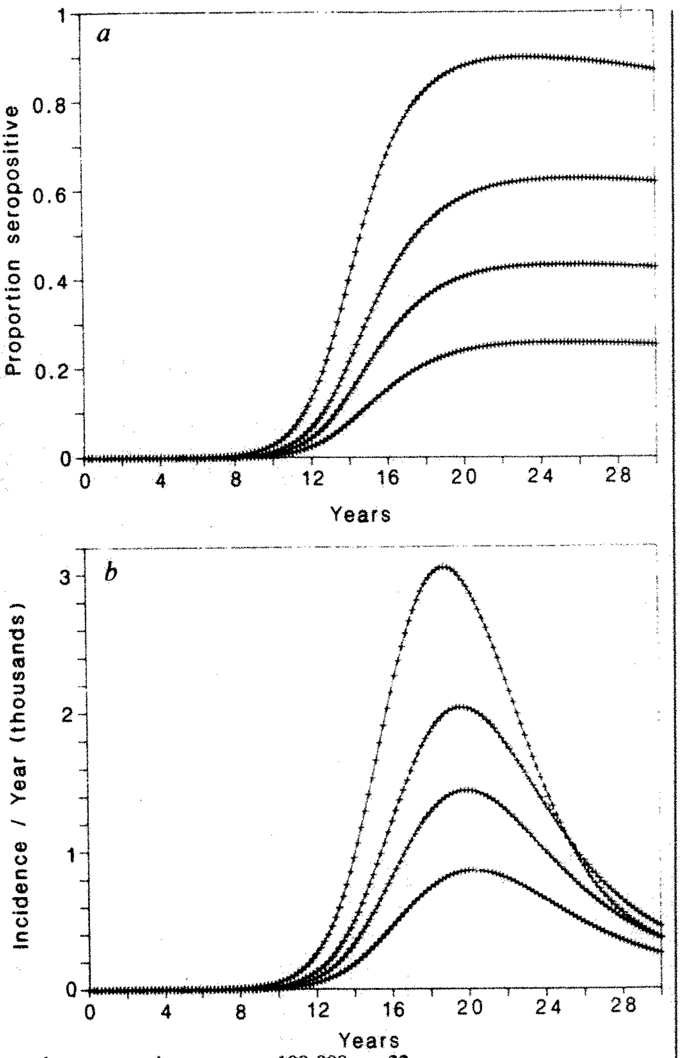

Fig. 6 The predictions of a model (see ref. 8) incorporating variable incubation periods, heterogeneity in sexual activity and recruitment of susceptibles. The two graphs record changes in seropositivity through time from the point of introduction of HIV into a community of 100,000 homosexual/bisexual males (graph *a*) and the incidence of AIDS yr^{-1} (graph b). Heterogeneity in sexual activity is described by a gamma distribution with a mean fixed at 5 partners yr^{-1} and variances 5, 25, 50 and 100 representing the predictions recorded by the four lines depicted in each graph. In *a* and *b* the smallest epidemic arises when the variance is largest and vice versa. Parameter values, $R_0 = 5$, $D = 5$ yr, $f = 0.3$ with the life expectancy of AIDS patient set at 1 yr from diagnosis and for the susceptible sexually active community at 32 yr from the point of joining the sexually active class. The 70% of infecteds who do not develop AIDS are assumed to be infectious for a period equal to D. The immigration of new susceptibles into the sexually active community was set at 100,000 per 32 yr.

must be based on the underlying epidemiological phenomena.

Heterosexual transmission

In developed countries, the extent to which HIV infection can be transmitted by heterosexual contacts is uncertain[43,48]. HIV infections in females come from contact with bisexual males (the dominant sexually-transmitted route at present), transfusion recipients, haemophiliacs and intravenous drug users[45]. If such females are not themselves a significant source of infection back into the homosexual/bisexual community (through contacts with uninfected bisexuals), we would expect the incidence of HIV infections among the female partners of bisexuals initially to rise roughly in proportion to the incidence among homosexual males.

Specifically, we would expect the ratio of HIV infection among female partners of bisexuals to that among bisexual males to be $\sim \beta'c'/\beta c$, where β and c are as previously defined, β' is the transmission probability for male-to-female contact, and c' is the mean number of new female partners acquired by a bisexual male, per unit time. We expect this ratio to be significantly less than unity, because β' is less than β, and c' significantly less than c. The data (Table 1) suggest the doubling time for heterosexually-transmitted HIV infection is roughly equal to that among homosexual males, which is in accord with our simple expectation.

As the epidemic progresses, a high proportion of homosexual males become infected (Fig. 2*e*). On the other hand, the pool of female partners for bisexual males may be large, and we would expect incidence of HIV infection still to be rising roughly linearly among women at and beyond the point at which saturation effects limit the epidemic among homosexual/bisexual males. How long this rise continues, and how many females are eventually likely to be infected, depends on the average duration of infectiousness in the transmitting group of males (which could be long if a substantial fraction remain asymptomatic carriers).

The transmission of HIV infection to females by bisexual males is a process whose initial dynamics is essentially determined by R_0 for transmission among homosexual males, thereafter the question of its transmission and maintenance by purely heterosexual contact arises. The basic reproductive rate for such heterosexual transmission of HIV, R_0', is given by

$$R_0' = (\beta_1\beta_2c_1c_2)^{1/2}D \qquad (7)$$

Here β_1 and β_2 are the transmission parameters for contacts between infected females and susceptible males and between infected males and susceptible females, respectively; c_1 and c_2 are as before given by (2), for the distribution in rates of acquiring new partners of the other sex by females and males, respectively.

Data are very limited on the transmission and sexual activity parameters, but the data in Fig. 4 suggest that c_1 and c_2 are significantly smaller than c among homosexual males. Further, it seems likely that $\beta_1 < \beta_2$ and that both are less than the MM for homosexual males. Thus overall, the factor $(\beta_1\beta_2c_1c_2)^{1/2}$ seems likely to be much smaller than c for homosexual males, which suggests that in developed countries, R_0 for purely heterosexual transmission is probably significantly smaller than R_0 for purely male homosexual transmission. Whether R_0 is greater than unity, such that HIV infection can maintain itself and spread by purely heterosexual transmission, is at present unclear. There is an urgent need for studies to measure c_1 and c_2 in different communities (stratified by age and social status) and to assess how these parameters change as a consequence of educational programmes and publicity campaigns on AIDS. The use of professional opinion poll organizations to gather quantitative data on rates of partner change over a series of specified time intervals by interview and questionnaire (Fig. 4*d*) could help to fill this gap in our knowledge, but estimates of β_1 and β_2 will come only from long term studies of the heterosexual partners of infected patients.

If R_0' does exceed unity, the incidence of HIV infection in the heterosexual community will initially grow exponentially, at a rate given by the analogue of equation (1):

$$\Lambda = (\beta_1\beta_2c_1c_2)^{1/2} - 1/D = (R_0' - 1)D \qquad (8)$$

The estimates above indicate that initial doubling times will be significantly longer than the 9 months or so for HIV among homosexual males; the slow initial growth will be difficult to discern against a background of homosexual transmission among males and bisexual transmission to females.

These observations are not necessarily inconsistent with the epidemiological situation for HIV in sub-Sahara Africa[6,49,56,57]. In contrast to the United States and the United Kingdom, where male/female ratios of AIDS cases have been of the order of 14:1 to 20:1, in certain parts of central Africa including areas in Zaire, Rwanda and Uganda, sex ratios approaching unity have been reported[45,50-53,56,57]. Very high prevalences of HIV antibodies have been found in males and females from surveys in urban and rural areas[52,56,57]. These points suggest that heterosexual transmission has been frequent in both directions and horizontal studies have shown that infection is associated with the age-related degree of sexual activity amongst heterosexuals[27,47,48,56,57]. We note, however, that in the early and approximately exponential phase of the epidemic, the ratio of the number of seropositive males to seropositive females is not unity, but is roughly $(\beta_1 c_1/\beta_2 c_2)^{1/2}$.

It is generally thought that β_1 is less than β_2 for HIV, although the facts are uncertain (for gonorrhea, for instance, male-to-female transmission, β_2 is roughly twice β_1). Obviously the average number of heterosexual partners of females and males, m_1 and m_2, are equal, but c_1 could significantly exceed c_2 if the variance of the distribution of rate of acquiring new sexual partners by females (associated with the concentrated activities of female prostitutes) is greater than that for males. This effect could partly offset β_1 being smaller than β_2. Although there is no *a priori* reason to expect the ratio $\beta_1 c_1/\beta_2 c_2$ to be exactly unity, its square root could easily be close to unity, which would explain the roughly equal proportions of seropositive males and females. Alternatively, the roughly equal proportions could be explained if homosexual transmission among males had coincidentally raised the seropositive proportion among males to around the level among females, or by transmission by contaminated needles in public and private medical services[6]. In any event, the rough equality of the seropositive proportions among males and females is a puzzle to be explained, and is not by itself evidence for purely heterosexual transmission

Discussion

The ideas presented above are based on relatively simple mathematical models, with the aim of making clear some of the essential relations between epidemiological parameters and the overall course of HIV infection within various populations. Such models help to clarify what kinds of epidemiological data are needed to make predictions. As such data become available, the models can be made more detailed and realistic.

For public health planning, the dominant unknown is f, the fraction infected who will eventually develop AIDS. Estimates of this parameter have been increasing in recent years, but on present evidence the possibility cannot be ruled out that it is as low as 20% or as high as virtually 100%. Thus any current predictions about the number of homosexuals likely to acquire AIDS are uncertain by at least a factor 5 or so. Better understanding of the mechanisms of interactions between virus and host may help to determine f, but it is possible that only epidemiological data gathered on a decade-long timescale, as cases accumulate, will resolve this question.

The duration of infectiousness, and the way this duration is distributed among different infectives, is also relevant to estimates of R_0 and thence of the eventual number infected; more studies directed towards eliciting this information, including looking for virus in the blood, excretions and secretions of infected individuals over time, together with longitudinal studies of the partners of infected patients, are needed[3].

More generally, there is need for more studies that combine information about the epidemiological history of individuals with information about their sexual habits, such as the important study by Winkelstein *et al.*[26] of an unbiased sample of homosexuals in San Francisco, which demonstrated the association between the number of sexual partners and probability of acquiring infection. We emphasize that what is epidemiologically important is the average rate of acquiring new sexual partners, not necessarily the same as the average number of partners per unit time. Some authors have recognized that sexually highly active individuals play a disproportionate role in the transmission dynamics; equation (2) quantifies this observation, making it clear that the epidemiologically relevant quantity is not the mean number of new partners but, rather, the mean-square divided by the mean.

In developed countries, at present and into the near future, it is probable that sexually-transmitted HIV infections among females are likely to come mainly from bisexual males. Whether subsequent spread of infection from such females to heterosexual male partners is likely to reach significant levels, and more importantly whether purely heterosexual transmission of HIV infection may be selfsustaining ($R_0' > 1$), depends on estimates of the transmission parameters β_1, β_2, c_1 and c_2.

We have shown how c for transmission among homosexual males can be estimated indirectly from data on initial doubling times, but corresponding estimates of $\beta_1 c_1$ and $\beta_2 c_2$ are much harder, partly because the corresponding doubling rates are likely to be longer and partly because these infections are likely to be masked by homosexual/bisexual transmission among males, and by bisexual-to-female transmission among females (both of which processes depend simply on c). Attempts to estimate these quantities directly, and thence to estimate R_0, are urgently needed.

From present knowledge, it is not possible to assess whether R_0' is greater or less than unity in developed countries, and it is thus not possible to say whether HIV infections could spread epidemically by purely heterosexual transmission. The evidence from Africa, however, clearly argues that the sexually active population as a whole should be regarded as at risk[6,47,56,57].

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Late Cretaceous and paroxysmal Cretaceous/Tertiary extinctions

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The various geological signatures at Cretaceous/Tertiary time including iridium and other associated elements, microspherules, and shock deformation features are compatible with the suggestion that the transition is marked by a period of intense volcanism. The volatile emissions from this volcanism would lead to acid rain, reduction in the alkalinity and pH *of the surface ocean, global atmospheric temperature changes, and ozone layer depletion. These environmental effects coupled with those related to the major sea level regression of the late Cretaceous provide the framework for an explanation of the selective nature of the observed extinction record.*

THE transition from Cretaceous to Tertiary 65 million years ago marked one of the major extinction periods in Earth history. The geological record contains evidence of many events at this time; thus it is not surprising that there has been considerable controversy about the causes of the extinctions. Any explanation must take into consideration the selective nature and chronology of these extinctions as well as whatever geological evidence is available to support ideas about the causes of the environmental changes. A provocative model was developed by Alvarez *et al.*[1] in support of the impact of an extraterrestrial object as the cause, based primarily on an excess of iridium in the vicinity of the boundary. This was a key observation and the model has stirred a great deal of interest and research on the subject. Although in a later paper Alvarez *et al.*[2] conceded the possible role of other factors, attention has been largely focused on the impact idea in discussing the mass extinction event, because of the iridium anomaly, and accordingly we concentrate on this.

Other geological evidence is very limited and the crater from the proposed impact has proved to be elusive. Furthermore, even if the iridium were of extraterrestrial origin, the dust cloud mechanism originally suggested as a cause for the extinctions would be more appropriate for total and geologically instantaneous extinctions rather than the selective extinctions observed which in most cases are distributed over a geologically significant time period. Therefore, it is useful to examine alternative models if these are plausible in terms of the record of the rocks and are more selective in their effects on organisms.

We suggest that there is strong evidence that the ultimate causative agent was not extraterrestrial but intrinsic to this planet. The argument is put forward that superimposed upon major environmental changes bound up with eustasy and climate over a longer time interval, there were significant environmental perturbations related to increased volcanism across the Cretaceous/Tertiary (K/T) boundary. In the following we first review the record of the faunal and floral changes that occurred. We next review the geological evidence of increased volcanism at K/T time and examine the environmental effects that might be expected from the volcanic emanations. We then discuss the faunal and floral changes that would be expected to have occurred as a result.

We wish to emphasize that this article predominantly advocates a particular point of view and may be criticized in giving less attention to alternative models. The calculations for the environmental effects of intense global volcanism are order of magnitude determinations. The intent has been to obtain reasonable estimates of these effects and to assess to what degree they, coupled with the anticipated effects of the Late Cretaceous sea level regression, may lead to an explanation for the difficult problem of both the selective nature of the K/T extinctions and the temporal sequence and intervals in which the extinctions occurred.

Fauna and flora at the end of the Cretaceous

The end of the Cretaceous is marked by the extinction of the marine reptiles, the flying reptiles, dinosaurs and ammonites together with numerous families of scleractinian corals, bivalves such as the inoceramids and rudists, gastropods and echinoids. In addition the coccolithophorids, planktonic foraminifera and belemnites suffered almost complete, though not geologically instantaneous, extinction with only a few species surviving the crisis; many genera of the larger benthic foraminifera and radiolaria also disappeared. On the other hand, and making due allowance for the poorer quality of the fossil record, a number of groups were little affected including many types of land plants, freshwater vertebrates, snakes, mammals and many marine invertebrates including deep sea benthic organisms[3]. However, a short-term perturbation at the species level at the horizon of the iridium anomaly seems to be recognizable locally in some invertebrate groups[2].

Extinction was particularly severe among planktonic organisms with calcareous skeletons, tropical reef invertebrates, and animals of large size in terrestrial communities. Tropical reef invertebrates were seriously affected but there are few data for the upper part of the Maastrichtian. Any extinction model should also take into account some further facts recognizable from the stratigraphic record. While the calcareous plankton extinctions apparently took place over a relatively short geological time interval of ~10,000 yr[4] and perhaps as little as 200 yr in some cases[5], other marine extinctions were more prolonged or took place earlier. Thus the ammonites had been in decline from the Campanian through the final, Maastrichtian, stage of the Cretaceous with the survivors of the decline being long-lived genera that had been relatively extinction resistant; the cause of the final extinction of these genera could well have been different from the cause of the more general late Cretaceous decline[6-8]. Although precise stratigraphic data are sparse, it appears that the most significant extinction event among shallow marine tropical benthic organisms such as rudists, hermatypic corals and large foraminifera was within the Maastrichtian, a few million years before the end of the Cretaceous[9]. As a consequence of late Cretaceous sea level regression, continuous shallow marine sections across the K/T boundary are rare and as a result it is not yet possible to determine if terminal Cretaceous benthic extinctions were on a global scale and therefore comparable to the contemporary event in the plankton.

Magnetostratigraphic and radiometric correlations indicate that extinctions of marine and terrestrial organisms were broadly contemporaneous but the precision is no better than a few hundred thousand years, and within terrestrial stratigraphic sections the last appearance of the dinosaurs and the floral K/T boundary are not coincident[10-14]. However, there is clearly a degree of inference involved in equating 'highest occurrence' in a stratigraphic section with 'last appearance' on earth. Changes at the K/T boundary in the angiosperm floras were relatively minor and geographically variable on a worldwide scale; they were preceded by a decline in diversity through the Maastrichtian[15]. Recent studies indicate that although some Cretaceous floral taxa became extinct, others were minimally affected at the K/T boundary and reappear in strata above[16].

Discussion of terminal Cretaceous extinctions has been dominated by the dinosaurs but Padian and Clemens[17] have pointed out that the terminal Cretaceous event may be nothing extraordinary in terms of rate or magnitude of extinction as compared with many extinction events earlier in dinosaur history, which is marked by high generic turnover rates of the order of a few million years or less. The significant difference therefore appears to be a fall in origination rate with the disappearing genera failing to be replaced. Furthermore, the latest report on dinosaur extinction in North America[18] suggests that it was a gradual affair which began 7 million years before the end of the Cretaceous and accelerated rapidly in the last few hundred thousand years during an interval of apparent competition from immigrant mammals. The last dinosaurs are claimed to be Palaeocene in age and not reworked from Maastrichtian strata, and they occur above the iridium anomaly horizon. Most groups of freshwater vertebrates (fish, amphibians, turtles, crocodiles) survived the K/T boundary relatively unscathed and among the terrestrial vertebrate faunas only the marsupial mammals were badly affected. Lizards were little affected and placental mammals were actually increasing in diversity before the dinosaurs became extinct.

Whatever was responsible for the extinction events that have been associated with the iridium anomaly, it appears to have been preceded by an increase in extinction rate among a number of groups spread over a period of up to several million years. Thus the impact extinction model is misleading in focusing attention solely on this event. Even for the paroxysmal K/T event in the marine stratigraphic record, there are evident complexities that have not yet been adequately discussed or evaluated.

In particular a model for the K/T extinctions should include processes that might in due course provide an explanation for the timing of various species extinction events within the K/T transition as well as the temporal duration over which a given event occurs. The specifics of timing within the K/T transition are not known in detail but it is known that variations exist[19]. For example, in the marine sedimentary record the change from Cretaceous to Tertiary nannofossils generally occurred over a measurable section interval of around 50–100 cm[4,20] though allowance must be made for bioturbational smearing. Planktonic foraminifera and nannoplankton (coccolithophorids) show a different extinction-recovery pattern at the K/T boundary. Whereas both groups are considerably reduced at the level of the iridium anomaly, the nannoplankton experienced final extinction well into the Tertiary[4,20,21]. The lack of precise correspondence between the foram and coccolith extinctions implies a more complex scenario than has been widely assumed.

Geological signatures at K/T boundary

Six years ago the interest of the geoscience community was stimulated by the discovery of an enhancement of iridium and other elements in clay layers at the K/T boundary in Italy and Denmark[1]. The iridium was interpreted to be of extraterrestrial origin from an asteroid impact, an instantaneous event in geological terms, and the clay layer to be the global fallout of impact generated fine dust which had been injected into the stratosphere. Subsequent studies negate both interpretations. There is no ubiquitous K/T clay layer. At the Danish section the clay layer (Fish clay) is of shallow water origin containing fish remains and showing strong dissolution features[22]. At the Italian section the clay layer is one of many clay layers in an alternating sequence of limestones and thin clay interbeds extending from Turonian to Eocene[23]. For most of the Deep Sea Drilling Project (DSDP) sections the K/T lithology is that of either a continuous calcareous sequence or a continuous pelagic clay sequence[19]. In Denmark the K/T Fish clay is smectite rich while in Italy the clay layers over a 3 m zone straddling the K/T boundary are enriched in kaolinite[24,25]. In the Caucasus the clay is predominantly palygorskite[26]; the clay mineralogy of the iridium-rich layer does not appear to be anomalous.

More recent and extensive iridium measurements show that the anomaly does not appear as a single spike in the record, indicative of an instantaneous event, but rather occurs over a measurable geologic time interval of 10 to 100 kyr or possibly longer[27]. For Italy there is a peak in the iridium anomaly for the K/T clay as well as enhanced level of iridium above background in the other clay layers extending over 1–2 m (100–200 kyr) both above and below the reference K/T clay as well as in the intervening limestones[28]. In Denmark similar results are found with enhanced levels of iridium extending into both the overlying Danian limestone and the underlying Maastrichtian chalk as well as in the Fish clay itself[29]. For Red Deer valley, Canada, the iridium flux interval is estimated to be 5–6 kyr[30]. All the DSDP sections that have been investigated also show an extended distribution of iridium at elevated levels of around 30 cm (30 kyr) at K/T time[31-34]. The most recent iridium investigations presented at the 1986 annual meeting of the Geological Society of America show multiple iridium anomalies for the K/T sections at Braggs, Alabama[35], Brazos river, Texas[36] and Haiti[37].

We suggest that these observations are best explained in terms of intense global volcanic activity over 10 to 100 kyr at K/T time. The extended iridium distribution is difficult to explain in terms of an instantaneous event either of extraterrestrial or terrestrial origin, and iridium enhancements are known to be associated with airborne particles from recent hot spot/mantle plume volcanic activity[38]. Further, the enhancements of other elements associated with the iridium anomalies are not in accord with an extraterrestrial origin; in particular the As/Ir and Sb/Ir ratios are three orders of magnitude greater than chondritic values but are in accord with a mantle origin[27].

There are other features associated with the K/T boundary. Microspherules are present. Some are of a K-feldspar composition, and these were originally interpreted to be high temperature droplets formed by an impact[39]. The K-feldspar has now been interpreted as a secondary, diagenetic replacement product[40], and microspherules of different chemical compositions are found at various K/T sections[41-44]. In Italy spherules were originally reported for numerous clay layers[45,46]. Some of the spherules, however, for one of the Italian sections (Bottaccione) are contaminant hollow spherules of a recent organic origin[47]. More recent analyses show that the solid spherules of K-feldspar or K-feldspar and glauconite composition for the Bottaccione section as well as for the Contessa section, which has no contaminant hollow spherules, have a concentration peak for the K/T clay as well as observable concentrations for other clay layers within 1–2 m above and below the reference K/T clay[43]. Microspherules of clearly volcanic origin are known to occur[48,49]. The volcanic microspherules are in the size range of 20–170 μm[48,49]; the K/T spherules studied at the Bottaccione and Contessa sections are in the size range 100–200 μm[43]. The present size of the K/T diagnetic replacement spherules may or may not represent their original size.

Shock deformation features in quartz have been found at a palynological K/T boundary in the western United States[50,51] and Canada[52] as well as 25 cm below this boundary at one location[51]. They have also been found at a K/T section in Italy[53] and in Austria[54]. Since shock metamorphic features are characteristic of materials associated with meteor craters[55], this finding was taken as supporting evidence for an impact origin for the K/T boundary events. A suggested candidate is the nearby Manson structure in Iowa[56], but this feature is apparently too small to account for the global K/T extinction events. On the other hand, if the quartz grains were transported out of the atmosphere and distributed around the world, as suggested in the dust cloud model, they would be expected to be annealed on their return to Earth, just as tektites are melted, and the shock features would not be retained. Shock features are also associated with the Vredefort intrusive and are interpreted to be of internal and magmatic origin there[57-59]. Recent studies have found shock metamorphic features in samples from the Toba caldera[60], Toba ash samples from Indian ocean deep sea cores[61], and Bishop tuff ash samples from the Long Valley caldera eruption[62]. Toba is the largest known volcanic eruption of the recent past, occurring about 75,000 years ago with a total eruption magnitude of about 400 times that of Krakatoa[63]. The eruption of Long Valley took place 600,000 yr ago producing pumice distributed throughout much of the western United States[64]. The indicated peak shock stresses for explosive volcanism could account for the microstructures observed at K/T time and a volcanic origin must be considered for them.

Several investigators have noted that the period of the late Cretaceous to early Tertiary also coincides with the emplacement of the bulk of the Deccan Traps. They are located in western India and are the largest continental flood basalt deposits of the past 200 Myr with an estimated volume of 1×10^6 km^3 (ref. 65). This number is probably on the low side as an estimate of their original volume for it does not include provision for postemplacement erosion nor their possible offshore extension to the west of India nor their possible extension to the east to include the Rajahmundry Traps[66]. The major episode of Deccan Trap emplacement has a potassium-argon age of 60-65 Myr[65,67]. The traps of this major episode were emplaced during a single reversed magnetic polarity interval[68-70]. Recent palaeomagnetic and radiometric data indicate that the bulk of the traps were emplaced during the K/T reversed magnetic polarity interval 29R (refs 71, 72). Magnetic interval 29R has a duration of about 600 kyr. At the time of their emplacement palaeogeographic reconstructions place the Deccan Traps in the vicinity of the Reunion hot spot[73].

In addition recent DSDP studies in the Walvis Ridge region of the south-east Atlantic show a distinct peak in explosive volcanism specifically at K/T time. The Walvis Ridge is a volcanic edifice presumed to have been formed by hot spot/mantle plume activity. The K/T cores are quite spectacular and unlike cores elsewhere in the geological section at these sites. Red to green bands (high ash fraction) alternate with white to light brown bands (low ash fraction); these are underlain by white Maastrichtian chalk at sites 527 and 529 and by Maastrichtian chalk interrupted by thick green to black volcanogenic sands at site 528 (ref. 74). The K/T boundary at these sites is characterized by major mineralogical and geochemical changes indicative of intense volcanic activity over a relatively short geological time interval of 300 kyr or longer[74,75].

Effects of volcanism at K/T boundary

As discussed above, the geological and geochemical signatures at K/T time indicate that there was intense global volcanism over a relatively short geological interval of around 10 to 100 kyr. A number of investigators have suggested that effects associated with intense volcanism over a geologically short time period were the causative agents for the K/T extinctions[22,27,45,76-87]. In particular, Keith[80,81] emphasized the importance of volatile emissions in the reduction of the atmospheric ozone layer and in changes in ocean chemistry, and McLean[82,83] and, Ekdale and Bromley[22] suggested that the carbon dioxide emissions are important to atmospheric and oceanic changes. Also, in conjunction with estimated effects from an asteroid impact, Lewis *et al.*[88] have emphasized the potential importance of acid rain toward explaining the scope and selectivity in biological extinctions at K/T time; in their model the acid rain would have been an instantaneous event. Prinn[89], in a more recent analysis, calculated that glancing impact of a comet could produce nitrogen oxides in the atmosphere sufficient to have a significant effect on organisms.

Table 1 Summary of values used in calculations

K/T iridium flux	63×10^{-9} g Ir cm^{-2}
Laki flux rates	7.17×10^6 t H_2SO_4 km^{-3} basalt 1.27×10^5 t HCl km^{-3} basalt
Kilauea flux rates	31 g Ir d^{-1} 10,500 t SO_2 d^{-1} 260 t HCl d^{-1} 11×10^6 m^3 basalt d^{-1}
Estimated K/T fluxes	17×10^{12} t H_2SO_4 27×10^{10} t HCl 11×10^6 km^3 basalt
Estimated K/T flux rates	$1{,}700 \times 10^6$ t H_2SO_4 yr^{-1} 270×10^5 t HCl yr^{-1} 1,100 km^3 basalt yr^{-1}
Earth area	510×10^6 km^2
Ocean area	360×10^6 km^2
Ocean volume	$1{,}370 \times 10^6$ km^3

The important consideration for evaluating the environmental effects of intense global volcanism is to have reasonable estimates for the volatile emissions particularly that of sulphur dioxide. We estimate these emissions directly from the observed iridium flux at K/T time and indirectly from the magnitude of Deccan volcanism.

Eruptions of basaltic magma release at least an order of magnitude greater SO_2 volcanic gas to the atmosphere than comparable volume eruptions of sililcic magma[90,91]. For the nonexplosive fissure eruption from Laki in 1983 Devine *et al.*[91] estimate fluxes of 7.17×10^6 tonnes H_2SO_4 km^{-3} basalt and 1.27×10^5 tonnes HCl km^{-3} basalt. Using the magnitude of the Deccan Trap flood basalts of 1×10^6 km^3 as an estimate of K/T volcanism total fluxes of 7.2×10^{12} t H_2SO_4 and 13×10^{10} tonnes HCl are obtained.

Alvarez *et al.*[92] have summarized the observed K/T iridium fluxes at 21 locations worldwide. The values range from 0 to 330×10^{-9} g Ir cm^{-2} with an average of 63×10^{-9} g Ir cm^{-2}. The total K/T iridium flux for the entire Earth is then $63 \times 10^{-9} \times 510 \times 10^6 \times 10^{10} = 32 \times 10^{10}$ g Ir, Table 1. Olmez *et al.*[93] have observed flux rates for various constituents including iridium from small vent eruptions of Kilauea. Their results are 31 g Ir d^{-1}, 10,500 t SO_2 d^{-1}, 260 t HCl d^{-1} and 11×10^6 m^3 basalt d^{-1}. Using these values and the K/T iridium value gives fluxes of $(32 \times 10^{10} \times 10{,}500/31) \times (98/64) = 170 \times 10^{12}$ t H_2SO_4, 270×10^{10} t HCl and 110×10^6 km^3 basalt. Olmez *et al.* note that the iridium flux shows a strong dependence on the temperature of the vent with an order of magnitude difference between the iridium released from the hotter vent as compared with the colder vent; they suggest that the iridium flux rate would be still higher for the temperatures of an erupting magma. Such higher release rates would proportionally lower the above estimates. Accordingly we estimate the K/T fluxes to be an order of magnitude less than the values calculated from the release vent data, or 17×10^{12}t H_2SO_4, 27×10^{10} t HCl and 11×10^6 km^3 basalt. This reduction brings the H_2SO_4 and HCl fluxes into reasonable agreement with the estimates from the data of Devine *et al.*, but there remains an order of magnitude difference in the total eruptive basalt between the two estimates.

The K/T iridium observations indicate a total temporal flux interval of 10 to 100 kyr. We do not know how the flux rate varied within this interval but the fact that it shows peaks indicates that it is reasonable to assume that there was considerable variation perhaps building to one or more peaks over shorter time intervals. Similarly from the Deccan Traps we know only that the bulk of the Traps were emplaced over a period of around 600 kyr but do not know how the eruption rate varied within this time interval. In the calculations that follow we have assumed that the fluxes were concentrated over a 10,000 yr interval rather than using a longer time estimate of 100,000 yr with superimposed peaks. This gives flux rates of $1{,}700 \times 10^6$ t H_2SO_4 yr^{-1}, 270×10^5 t HCl yr^{-1} and 1,100 km^3 basalt yr^{-1}.

As with present day fossil fuel emissions, the SO_2 injection into the atmosphere by K/T volcanism will return to the ground or enter the ocean as acid rain. The 1976 fossil fuel emissions of SO_2 were 28×10^6 t yr^{-1} for the United States and 53×10^6 t yr^{-1} for Europe, giving an equivalent H_2SO_4 rain of $(28+53) \times 10^6 \times (98/64) = 120 \times 10^6$ t yr^{-1} (ref. 94). The K/T volcanic acid rain would be $1{,}700/120 = 14$ times greater. This is a global average value. The acid rain for any given location or time could be substantially in excess of this value. If we project from the recognized effects of present day acid rain, it seems apparent that the greater concentrations at K/T time would have a significant effect on terrestrial fauna and flora.

One of the more important biochemical properties of the ocean is its buffering capacity. It takes a large amount of hydrogen ion input to significantly alter ocean alkalinity. Ocean alkalinity is defined as $A = HCO_3^- + 2CO_3^{2-}$. For the upper ocean above the main thermocline at around 100 m, the bicarbonate ion concentration is around 1.8 g-at m^{-3} and the carbonate ion concentration around 0.25 g-at m^{-3} for an alkalinity of 2.3 g-at m^{-3} (ref. 95). For the deep ocean the alkalinity is around 2.5 g-at m^{-3}. The titration effect of the H_2SO_4 hydrogen ion flux from K/T volcanism will be first to reduce all the available carbonate ion to bicarbonate ion and then to reduce the bicarbonate ion to carbonic acid[96]. For a reduction of all the carbonate ion in the upper ocean layer, the equilibrium between the surface ocean and the atmosphere would lead to a corresponding reduction in *p*H from a normal value of 8.2 to around 7.4 or possibly less[97].

The total hydrogen ion flux to the ocean from K/T volcanism will be $17 \times 10^{12} \times 10^6 \times (2/98) = 35 \times 10^{16}$ g-at. This flux will lead to a gradual reduction in alkalinity for the whole ocean by up to an amount of $35 \times 10^{16}/1370 \times 10^6 \times 10^9 = 0.26$ g-at m^{-3} over the 10,000 yr period. An alternative approach is to consider flux rates into and out of the upper ocean above the main thermocline with the deep ocean as an infinite sink. The hydrogen ion flux rate will be $1{,}700 \times 10^6 \times 10^6 \times (2/98)/360 \times 10^6 \times 10^6 = 0.10$ g-at m^{-2} yr^{-1}. For an upper ocean thickness of 100 m and a residence time of 50 yr[95], the reduction in alkalinity would be $0.10 \times 50/100 = 0.05$ g-at m^{-3}. The first calculation does not include time-dependent feedback effects which would reduce the estimate. The second calculation does not include hydrogen ion exchanges from the deep ocean which would increase the estimate, and palaeoceanography suggests that vertical circulation of the ocean was more sluggish at K/T time[98] which would also increase the estimate. Within these constraints it does appear that the K/T hydrogen ion flux to the ocean would be sufficient to product a noticeable change in *p*H particularly for the surface ocean waters.

Global cooling of Earth surface temperature is a known effect from large volcanic eruptions[90,99-102]. The observed cooling is related to the volcanic aerosols, principally SO_2, which have a stratospheric residence time of 1-2 yr as compared with the silicate dust which falls out within a few months[90,103-108]. While most of the observations have been of explosive volcanoes, Stothers *et al.*[109] and Rampino *et al.*[110] have calculated that large volume fissure eruptions are capable of injecting large quantities of sulphate aerosols into the lower stratosphere. From the time dependent relation for aerosols in the atmosphere given by Hansen *et al.*[103], the average residence time is 1.2 yr. The amount of SO_2 in the atmosphere from K/T volcanism would be $1{,}700 \times 10^6 \times 1.2 \times (64/98) = 1{,}300 \times 10^6$ t. Extrapolating from the empirical relation between observed surface cooling and sulphur emissions from large historic eruptions given by Devine *et al.*[91], a global cooling of 3.1 °C would be expected. It is important to emphasize that this cooling estimate is an extrapolation from the effects of known volcanic eruptions and that it presumes the validity of the calculations for large volume basaltic fissure eruptions of Stothers *et al.*[109] and Rampino *et al.*[110]. One of the anticipated effects of this cooling would be that the replacement species following an extinction would be of a higher latitude and hardier form than their predecessors.

HCl aerosols injected into the stratosphere from historic volcanic eruptions have led to a decrease in the protective ozone layer[111-113]. In discussing the effects of the Krakatoa and Agung eruptions Stolarski and Butler[113] predict a global ozone layer depletion of 7.7% for a stratospheric injection of 300×10^6 kg HCl. For the HCl produced from K/T volcanism the average atmospheric content would be $270 \times 10^5 \times 10^3 \times 1.2 = 32{,}000 \times 10^6$ kg HCl. This is $32{,}000/300 = 110$ times greater than that for a 7.7% reduction in the ozone layer and would suggest a serious depletion of the ozone layer at K/T time. The principal biological effect of the resultant increase in ultraviolet radiation would be on those terrestrial animals that were unprotected or unable to hide in either their adult or reproductive stages.

Taking a value of 300 p.p.m. for carbon released as CO_2 from volcanic eruptions[114] and a density of 2.8 g cm^{-3} for basalt, the K/T carbon flux to the atmosphere will be $300 \times 10^{-6} \times 2.8 \times 1{,}100 \times 10^{15} = 0.9 \times 10^{15}$ g yr^{-1}. The present anthropogenic flux of fossil fuel carbon emissions to the atmosphere is 5×10^{15} g yr^{-1} and that from terrestrial biota 53×10^{15} g yr^{-1} (ref. 115). McLean[83] argues that the cumulative effect of volcanic CO_2 over 500,000 yr can be substantial although feedback mechanisms over that time period could seriously affect this model. In any event the effect of increased CO_2 would also be to reduce ocean alkalinity.

The sulphate ion input to the ocean from K/T volcanism will be $17 \times 10^{12} \times (96/98) = 17 \times 10^{12}$ t. The sulphate ion concentration in the ocean is 28 g-at m^{-3} (ref. 95), giving a total sulphate content for the whole ocean of 3.7×10^{15} t. The K/T volcanic sulphate flux to the ocean is small compared with the ocean reservoir.

An additional consideration is what global geological record such a period of increased volcanism might leave. Here we wish to consider the global record exterior to the immediate regions of basalt or volcanic ash emplacement. Deirmendjian[116] estimated that the explosive volcanic eruption of Krakatoa injected an amount of fine dust into the atmosphere of 0.17% of the total eruption volume. For K/T volcanism we have presumed that the bulk of the volcanism was of a basaltic type. If we assume that 1/4 of the K/T volcanism was explosive a global fine dust layer of $11 \times 10^6 \times 0.0017 \times 10^5/4 \times 510 \times 10^6 = 0.9$ cm would result. The global record of K/T volcanism would be small although local effects could be significant.

An extinction model

We have suggested that a substantial increase in volcanism at K/T time would produce acid rain, decrease in surface ocean alkalinity, global temperature change and ozone layer depletion. In addition there is associated with the late Cretaceous a pronounced regression of the sea lasting for a period of a few million years[117,118]. Evidence for this regression is primarily stratigraphic[117-119] but it is also supported by geochemical data[120,121]. The seawater ratio of $^{87}Sr/^{86}Sr$ shows a gradual increase in the late Cretaceous continuing into the Tertiary, interrupted by a sharp rise and fall at the end of the Cretaceous. Rise in the ratio relates to increase in continental runoff and is most plausibly explained in terms of increased continental area due to sea level fall[117,122]. Regression of the sea during the late Cretaceous would also affect climate. Pitman[123] explains the sea level regression in terms of ocean spreading ridge volume changes which are related to changes in the rate of seafloor spreading. Other possibilities related to plate tectonic activity include change in the length of the ocean ridge system and subsidence of intraplate volcanic mountains in the Pacific[117]. We consider that the major regression of the sea during the late Cretaceous, explained in terms of plate tectonics, and the K/T volcanism, explained in terms of hot spot/mantle plume activity, must be generically related.

The crux for any scenario of late Cretaceous and K/T environmental events is how well it explains the faunal and floral changes that we see in the geologic record. It is not only important that the model provide an explanation for those species that became extinct; it must also account for those which showed little or no change across the K/T transition. We list below the observed phenomena together with suggested causes in terms of the above factors.

The carbonate dependent planktonic organisms, foraminifera and coccolithophoridae, of the surficial ocean waters show major extinctions at K/T time[20,124,125]. While both groups declined abruptly at the end of the Cretaceous, the main coccolith extinctions occurred a few thousand years later[4,21]. Modern planktonic foraminifera become inhibited below a pH of 7.6–7.8 (refs 83, 126, 127), and modern coccolithophoridae do not survive below a pH of 7.0–7.3 (refs 83, 128–130). If K/T planktonic forms were similarly affected, the changes in pH resulting from volcanism at K/T time would lead to extinction effects for both foraminifera and coccolithophoridae with the foraminiferal extinctions preceding those of the coccoliths.

The earliest Tertiary carbonate dependent plankton show a change from large and ornate forms to small and simple forms[4,131]. For modern plankton small forms survive better in a lower pH environment than large forms[127], and an environment which is undergoing change is selective toward smaller species as compared with a more stable environment[132]. The anticipated pH and environmental conditions for the K/T surface ocean waters would be preferential to the smaller forms.

The earliest Tertiary calcareous micro and nannofossil replacement forms are of a higher latitude, colder water type than their predecessors[4]. Higher latitude forms are generally more robust than their warm water counterparts. We would expect that the major effects of decreased pH would be in the tropical and temperate surface waters and that any effects of global cooling would favour survival of the higher latitude and hardier forms.

Dinoflagellates are the least affected of the plankton across the K/T transition[125,133,134]. Diatoms, silicoflagellates and radiolarians are not as numerous in Upper Cretaceous and Lower Tertiary sediments as the dinoflagellates, and tabulations differ as to the changes in these planktonic forms[125,134]. Dinoflagellates are not dependent on carbonate. In freshwater acid lakes with a pH of 4.0–5.0 dinoflagellates are one of the few surviving planktonic groups[135]. We would expect that the pH conditions of the K/T surface ocean waters would have less effect on the dinoflagellates than on the carbonate dependent plankton.

Deep water benthic foraminifera in the oceans show little change across the K/T transition[124,125,133]. It would be expected that alkalinity and pH changes would be greatest in the surface waters especially if the Cretaceous ocean were more strongly stratified than the modern ocean as would appear to have been the case[98]. We would expect that the K/T environmental changes would be smaller for the deep ocean with less effect on the benthic foraminifera.

There is a peak in calcite dissolution for shallow water forms at K/T time[22,136], and some DSDP sites show evidence of dissolution of some of the micro and nannofossils[4]. The former would be expected for the anticipated changes in alkalinity of the upper ocean and shallow waters; the latter may be related to changes in the calcite compensation depth associated with the anticipated smaller decrease in deep ocean alkalinity.

For shallow water macrofauna the most striking changes took place a few million years before the end of the Cretaceous[7–9]. Shallow water macrofauna and especially reef communities would be expected to be affected by sea level changes. While there is evidence of extinction at the boundary (and the problem of lack of complete sections across the boundary in shallow water facies has already been alluded to), it could relate to a collapse of the food web and requires no special explanation. Eurytopic shallow water benthos that had survived the earlier perturbations of the physical environment that led to the extinction of the rudists and other stenotopic groups would be expected to be relatively resistant to extinction. The key issue for the marine realm therefore concerns the plankton.

There is dispute about whether the dinosaurs had already been in decline prior to when they finally became extince[14,18,131,137–140]. What should not be in dispute is that, as generally large organisms with small populations, dinosaur species were probably always relatively vulnerable to extinction; and the importance of the terminal Cretaceous period lay primarily in the failure of new dinosaur groups to evolve and occupy the niches vacated by the extinct forms, as had happened many times previously in the Mesozoic. We speculate, as have several other investigators[18,137,138,140], that sea level regression led to an increase in continental type climates with greater seasonal extremes of temperature. This environmental stress may possibly have been reinforced by the increase in ultraviolet radiation at K/T time.

Placental mammals, birds, and freshwater amphibians and reptiles survived the K/T transition with little to no change[137–141] which implies that changes in the continental environment were somewhat less drastic for many groups. It is difficult to perceive a common protective element for such diverse organisms. Different factors may be speculated upon for different cases, for example, large population size, wide temperature tolerance, and escape from ultraviolet radiation by nocturnal habits and subaquatic or subterranean habitats.

Freshwater fish showed selective extinctions[139]. These changes may be related to the effects of acid rain. Modern freshwater fish in a Canadian lake which has undergone severe changes in pH from acid rain show selective extinctions with, for example, walleye and lake trout going out at a pH of 5.8–5.2, northern pike and white sucker going out at a pH of 5.2–4.7, and yellow perch and lake herring surviving at a pH of less than 4.7 (ref. 142). If these examples apply in principle to K/T freshwater fish, extinctions of a selective nature would be expected.

Terrestrial floral changes were also selective and occurred over an extended time interval[11,15,16,140,143]. At the iridium anomaly horizon in North America, however, there is a sharp palynological change indicative of a decline of angiosperms at the expense of ferns[144]. Hickey[15] suggests that climatic changes during the late Cretaceous may be the important longer term environmental factor but it is also possible that acid rain at K/T time may have been a contributive factor to account for the shorter term floral changes[16,144]. Modern flora in Canada from regions subject to acid rain show selective effects with, for example, white pine and white birch being sensitive, red pine and balsam fir showing intermediate effects, and red cedar and sugar maple being tolerant[145]. If similar species tolerance existed at K/T time, extinctions of a selective nature would be expected.

Conclusions

The selective nature of the extinction record during the late Cretaceous and at K/T time does not support a model which calls upon a decrease in solar radiation due to dust in the atmosphere or stratosphere, and no other comprehensive scenario solely related to impact has been proposed. We have proposed a scenario that calls upon a substantial increase in volcanic activity over at least 10,000 yr at K/T time. The volatile emissions from this volcanism would lead to acid rain, reduction in the alkalinity and *p*H of the surface ocean, global atmospheric cooling, and ozone layer depletion. These effects coupled with those associated with the major sea level regression of the late Cretaceous provide the framework for a reasonable explanation of the extinction record.

The mass extinction of calcareous plankton, which is a more striking and enigmatic phenomenon than the more widely publicized extinction of the dinosaurs, together with correlative extinctions in other groups may be viewed as an environmental *coup de grace* following a longer period of increased environmental stress associated with reduced habitat area and a deteriorating temperature regime.

Note added in proof: We note two recent studies related to K/T events. H. J. Hansen *et al. Bull. geol. Soc. Denmark* **35**, 75-82 (1986), examined microspherules from Denmark, New Zealand and Spain. The microspherules are of a geothite or K-feldspar and goethite composition. After dissolution in HCl and HF, organic spheres were found which according to their morphology and structure appear to belong to the green algal group *Prasinophyta*. The authors conclude that the microspherules are the result of diagenetic infill of prasinophyte algae. D. E. Loper and K. McCartney *Geophys. Res. Lett.* **13**, 1525-1528 (1986) and P. Olson *et al. Nature* (in the press), conjecture that episodes of volcanism including that at K/T time are related to instabilities in the thermal boundary layer at the base of the mantle and that these instabilities produce increased mantle plume activity on a global scale.

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ARTICLES

Molecular cloning of a protective antigen of schistosomes

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The complementary DNA sequence encoding the M_r *28,000 antigen of* Schistosoma mansoni *has been isolated and expressed in* Escherichia coli. *Experimental vaccination of rats, hamsters and monkeys with a recombinant fusion protein induces a strongly cytotoxic antibody response. Immunization of rats and hamsters with this protein leads to significant protection against a natural challenge infection with live cercariae.*

SCHISTOSOMIASIS is a chronic debilitating disease affecting human beings throughout 70 countries in several parts of the world. It is estimated that 200-300 million people are infected by various species of schistosomes. In spite of the development of active and relatively safe drugs, prevention of rapid reinfection has remained a problem, requiring repeated drug applications over a long period. There is a clear need therefore for alternative control measures and in particular for an effective vaccine inducing significant levels of protection against the invasive stage of the parasite.

Following the identification of potential mechanisms of immunity in man and experimental animal models, several schistosomal antigens have been characterized and their protective activity assessed either by passive transfer of the corresponding antibodies or by direct immunization with purified antigenic preparations. Passive transfer of immune sera[1,2] and experimental vaccination using either immunoaffinity purified antigens[3] or gel filtration purified proteins[4,5] have been shown to induce statistically significant levels of protection.

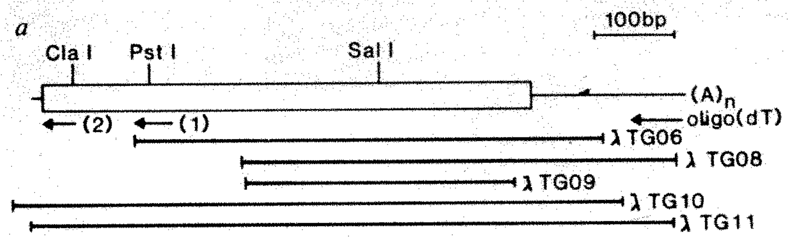
We have described a *Schistosoma mansoni* antigen with an apparent relative molecular mass of 28,000 (M_r 28K) which could be detected on the schistosomulum surface and was among the *in vitro* translation products from larval and adult stage RNA[6]. Several groups[7-9] have identified cloned molecules of 27-28K but there is no evidence of identity with the molecule described in this paper. A significant level of protection was achieved by immunization of Fischer rats and BALB/c mice with a gel purified protein fraction of M_r 28K (ref. 10).

Here we report the cloning and expression in *E. coli* of the complementary DNA encoding a 28K antigen present in extracts of adult worms. Immunization of rats, hamsters and monkeys with a recombinant protein containing the major epitopes of the native 28K antigen induces high levels of serum cytotoxicity towards schistosomula. In addition, rats and hamsters have been shown to be protected against a challenge infection to a degree comparable to that observed after immunization with the native molecule. The 28K antigen was also shown to be present in extracts of related species like *Schistosoma bovis*, *S. haematobium* and *S. japonicum*, demonstrating that an effective vaccine against both bovine and human schistosomiasis could be elaborated.

Cloning the cDNA encoding the 28K antigen

Total RNA from adult worms was used to synthesize double-stranded cDNA. Following nuclease S1 treatment, the cDNA was fractionated on linear sucrose gradients, and the size fractions between 500 and 4,000 base pairs (bp) were pooled. Extremities were extended with 10-15 dG residues and DNA was inserted into λgt10[11] using the synthetic adapter 5'-AATTCCCCCCCCCCC-3'[12]. Phages containing inserts were selected and amplified on *E. coli* POP101[13], resulting in a library of 2×10^6 independent recombinants. Phage DNA was prepared after two CsCl equilibrium centrifugations and inserts obtained after *Eco*RI digestion were recovered from sucrose gradients by pooling the size fraction between 500 and 2,000 bp. DNA was ligated into the *Eco*RI site of the β-galactosidase gene present in λgt11, packaged *in vitro* and samples of the library containing a total of 1×10^8 independent recombinants were plated directly on *E. coli* Y1090.

Screening was done at high density using both rat and rabbit polyclonal monospecific antibodies against the 28K antigen. Replicates were developed by subsequent incubation with biotinylated anti-rat or anti-rabbit antibodies and a biotin-peroxidase complex. Final staining in the presence of a colour reagent resulted in clearly detectable signals. From a first screening, three independent candidates λTG06, λTG08 and λTG09 were selected for further characterization by sequencing the cDNA inserts in M13 derivatives using the chain-termination reaction. An oligonucleotide was synthesized based on the sequence of the cDNA insert of λTG06 and used to screen the λgt10 library made with RNA from adult worms. Two additional candidates, λTG10 and λTG11, were isolated and sequenced as above. All five cDNA inserts contained overlapping sequences (Fig. 1). Assembling individual fragments gave a longest possible cDNA of 783 bases followed by a poly(A) track. Translation of the sequence starting with a Met residue at nucleotide position 7, gave a predicted protein of 211 amino acids with M_r 28,000.



b

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                                                31                                                         61                                                       ↓
TGCAAG ATG GCT GGC GAG CAT ATC AAG GTT ATC TAT TTT GAC GGA CGC GGA CGT GCT GAA TCG ATT CGG ATG ACT CTT GTG GCA GCT GGT
       Met Ala Gly Glu His Ile Lys Val Ile Tyr Phe Asp Gly Arg Gly Arg Ala Glu Ser Ile Arg Met Thr Leu Val Ala Ala Gly

91                                   121                                     151
GTA GAC TAC GAA GAT GAG AGA ATT AGT TTC CAA GAT TGG CCA AAA ATC AAA CCA ACT ATT CCA GGC GGA CGA TTG CCT GCA GTG AAA GTC
Val Asp Tyr Glu Asp Glu Arg Ile Ser Phe Gln Asp Trp Pro Lys Ile Lys Pro Thr Ile Pro Gly Gly Arg Leu Pro Ala Val Lys Val
                                                                                                [Leu Pro Ala Val Lys]
181                                  211                                     241
ACT GAT GAT CAT GGG CAC GTG AAA TGG ATG TTA GAG AGT TTG GCT ATT GCA CGG TAT ATG GCG AAG AAA CAT CAT ATG ATG GGT GAA ACA
Thr Asp Asp His Gly His Val Lys Trp Met Leu Glu Ser Leu Ala Ile Ala Arg Tyr Met Ala Lys Lys His His Met Met Gly Glu Thr
                                                                        [Tyr Met Ala Lys]
271                                  301                                     331
GAC GAG GAA TAC TAT AGT GTT GAA AAG TTG ATT GGT CAG GCT GAA GAT GTA GAA CAT GAA TAT CAC AAA ACT TTG ATG AAG CCA CAA GAA
Asp Glu Glu Tyr Tyr Ser Val Glu Lys Leu Ile Gly Gln Ala Glu Asp Val Glu His Glu Tyr His Lys Thr Leu Met Lys Pro Gln Glu

361                                  391                                     421
GAG AAA GAG AAG ATA ACC AAA GAG ATA TTG AAC GGC AAA GTT CCA GTT CTT CTC AAT ATG ATC TGC GAA TCT CTG AAA GGG TCG ACA GGA
Glu Lys Glu Lys Ile Thr Lys Glu Ile Leu Asn Gly Lys Val Pro Val Leu Leu Asn Met Ile Cys Glu Ser Leu Lys Gly Ser Thr Gly

451                                  481                                     511
AAG CTG GCT GTT GGG GAC AAA GTA ACT CTA GCT GAT TTA GTC CTG ATT GCT GTC ATT GAT CAT GTG ACT GAT CTG GAT AAA GGA TTT CTA
Lys Leu Ala Val Gly Asp Lys Val Thr Leu Ala Asp Leu Val Leu Ile Ala Val Ile Asp His Val Thr Asp Leu Asp Lys Gly Phe Leu

541                                  571                                     601
ACT GGC AAG TAT CCT GAG ATC CAT AAA CAT CGA GAA AAT CTG TTA GCC AGT TCA CCG CGT TTG GCG AAA TAT TTA TCG AAC AGG CCT GCA
Thr Gly Lys Tyr Pro Glu Ile His Lys His Arg Glu Asn Leu Leu Ala Ser Ser Pro Arg Leu Ala Lys Tyr Leu Ser Asn Arg Pro Ala
                                           [ *  Asn Leu Leu Ala  *   *  Pro  * ]
631                                  661                                     691
ACT CCC TTC TAA ACC TAT CAA CAG AAA GCT CGG TGT AAC GAG ATT TAA GAT ATT GAT AGT AAA GGA CTA GTG TCA CCT TTT TAC AAG GAT
Thr Pro Phe ***

721                           751                           781                           811
GTCATTTGTTTTATGGGTGTTTTTTTCGCAATTGTTATTAAAATTAACTTAGTTTCCTGTTTAAAAA
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Fig. 1 *a*, Positions of the independent cDNAs relative to the presumptive messenger coding for a 28K protective antigen of *S. mansoni*. Arrows (1) and (2), synthetic oligonucleotides. *b*, Complete structure of the assembled cDNA encoding a 28K protective immunogen present in total soluble extracts of adult worms. The predicted protein sequence is shown below the DNA. Arrow, cleavage site for a putative signal peptide. The sequence of three peptides, obtained after a tryptic digest on the native protein (see Fig. 2), could be matched with the amino-acid sequence deduced from the cDNA (boxes; *, unidentified residue).

Methods. Recombinant phages λTG06, λTG08 and λTG09 originate from an adult worm λgt11 library which was screened with a polyclonal rat or rabbit antibody raised against the native antigen eluted from SDS-polyacrylamide gels. The two cDNAs, λTG10 and λTG11, containing a complete copy of the coding region, were isolated after rescreening a λgt10 library with the synthetic oligomer (1) complementary to the region corresponding to base pairs 135–152. The 5′ start of the mRNA was mapped by primer extension with oligonucleotides (1) and (2) on RNA of adult worms. Oligonucleotides were kinated to a specific activity of 5,000 Ci mmol^{-1} using [γ-^{32}P]ATP and T4-polynucleotide kinase, annealed with poly(A)$^+$ RNA and extended by incubation with M-MLV transcriptase. Reaction products were analysed on a denaturing sequencing gel relative to an end-labelled *Hpa*II digest of pBR322, resulting in transcripts of 63 bases for primer (2) and 165 bases for primer (1). DNA sequencing of restriction fragments subcloned in M13 derivatives was by the chain termination method[18] with both M13 and internal primers. Sequence data were confirmed in both directions, preferentially using the cDNA insert of independent λ isolates.

The primary structure of this antigen was confirmed by partial protein sequence analysis of the native 28K antigen eluted from SDS-polyacrylamide gels and purified to homogeneity by reverse-phase HPLC. A trypsin digest of this material and separation of the cleavage products resulted in a complex pattern of peaks (Fig. 2). The sequence of three of the tryptic fragments could be matched with the corresponding peptide regions present in the amino-acid sequence predicted from the cDNA.

From primer extension experiments (Fig. 1) on RNA of adult worms it could be deduced that the cap-site of the messenger was located only 14 nucleotides upstream of the first base as given in Fig. 1. It seems therefore very likely that translation is initiated from the first ATG-codon at nucleotide position 7. The presence of a potential cleavage site for a signal peptide can be deduced by comparison with the empirical rules[14], which suggest cleavage after residue 27 (Ala). However, the expression in *E. coli* of the complete protein containing 211 amino acids (data not shown) gave a band on SDS-polyacrylamide gel electrophoresis (PAGE) gels which migrated at an identical position relative to the native protein. We therefore suggest that this particular antigen is not secreted by the organisms following the classical pathway described for many other surface antigens. It would appear that this 28K antigen of *S. mansoni* is not glycosylated, as no potential *N*-glycosylation sites are present in the predicted amino-acid sequence, confirming previous observations that the size of the *in vitro* translation product is identical to the size of the native protein. The frequency of this gene in the λgt10 and λgt11 libraries, 2×10^{-4} and 5×10^{-5} respectively, was certainly lower than expected assuming that this component represented around 1% of the proteins in total extracts of adult worms.

Experimental immunization

The method adopted for isolating this antigen presumably selected those parts of the mature 28K antigen which contained the major epitopes recognized by the host immune system. Compar-

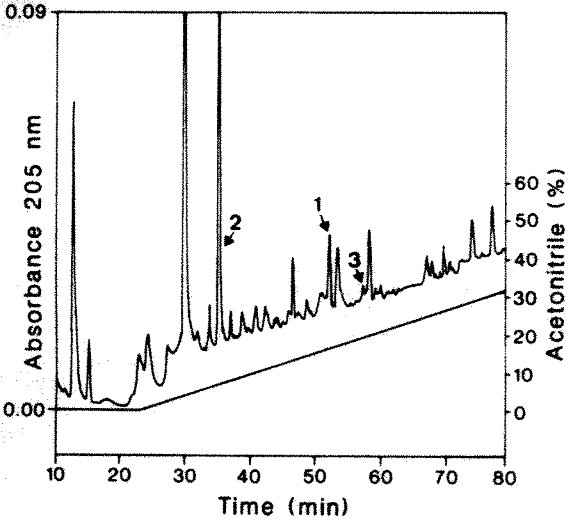

Fig. 2 Partial sequence analysis the native 28K antigen eluted from SDS-PAGE gels. Sequences of arrowed peptides as follows: 1, Leu-Pro-Ala-Val-Lys; 2, Tyr-Met-Ala-Lys; 3, Xxx-Asn-Leu-Leu-Ala-Xxx-Xxx-Pro-Xxx; Xxx is an unidentified residue.

Methods. Adult worms of *S. mansoni* were homogenized in phosphate-buffered saline (PBS) and soluble antigens were suspended on preparative SDS-PAGE gels. The fraction corresponding to a M_r of 28K was electro-eluted, dialysed against water and concentrated by lyophilization. The sample was made up in 20% in 1-propanol and purified by HPLC chromatography on a Vydac C18, 10 μm column (reference 201 TP54, The Separations Group), equilibrated in 0.1% trifluoroacetic acid. The column was eluted with a 20–60% gradient of 1-propanol over 60 min and fractions containing the immunogenic 28K component were identified by Western blotting with a polyclonal rabbit antibody raised against the 28K gel fraction of adult worms. Protein (25 μg) was lyophilized and resuspended in 8M urea, diluted five times in 0.1M *N*-ethyl morpholine acetate, 0.1 mM $CaCl_2$, *p*H 8.3, containing 1.25 μg of L-1-tosylamide-2-phenyl-ethylchloromethyl ketone (TPCK)-trypsin (Worthington). Sample was digested for 4 h at 37 °C and the reaction was stopped by adding trifluoroacetic acid to 1%. After lyophilization, the material was resuspended in 0.1% trifluoroacetic acid and separated on a Vydac C18, 5 μm column (reference 218TP54, The Separations Group), equilibrated in 0.1% trifluoroacetic acid and developed with a 0–60% gradient of acetonitrile in 120 min. Peptides labelled with a number in the chromatogram were loaded on a gas phase sequenator (model 470, Applied Biosystems) and phenylthiohydantoin amino acids were identified by on-line analysis (model 120, Applied Biosystems).

ing the position of the different cDNA inserts (Fig. 1), it can be concluded that the major antigenic epitopes in this molecule are located between amino-acid residues 91 and 200.

A recombinant antigen containing the essential part of the adult *S. mansoni* 28K antigen was expressed in *E. coli* as a cII fusion protein. Appropriate cDNAs originating from λgt11 candidates were inserted directly, into a P_L-expression vector[15] using the unique *Eco*RI site located 14 amino acids downstream of the *cII* initiation codon. This construction, containing the insert from λTG06, directed the synthesis of a fusion protein with M_r 25K containing 172 amino acids of the C-terminal region of the native 28K antigen.

Cultures were induced for the synthesis of this fusion protein by incubation at 42 °C and extracts were analysed on Coomassie-Blue stained SDS-PAGE gels. A protein band with M_r 25K was observed, most of this material being present in the non-soluble fraction obtained after lysis of the cells by sonication. The protein was recovered with a minimum purity of 80% by solubilization in the presence of 0.2% SDS, giving a solution of about 1 mg ml^{-1} contaminated with small amounts of *E. coli* membrane protein components. A sample of this preparation was analysed by Western blotting (Fig. 3*a*), indicating that the fusion protein was efficiently recognized either by the rat or the rabbit antisera raised against the native antigen. The biological property of the 25K fusion protein was tested by experimental vaccinations of rats and hamsters using 50 μg of the solubilized protein in the presence of aluminium hydroxide adjuvant. Animals received a second dose two weeks later by intraperitoneal injection and sera were collected starting from day eight. Rat samples were tested on Western blots for the presence of specific antibodies against the native 28K antigen of *S. mansoni* (Fig. 3*b*). The maximum response, observed after three weeks, reached levels comparable to those observed with sera raised against the native 28K protein. Note that extracts of the related species *Schistosoma bovis*, *S. japonicum* and *S. haematobium*, showed the presence in adult worm antigen of identical epitopes which were recognized by the rat serum against the fusion protein of M_r 25K (Fig. 4). Control immunizations with SDS-extracted membrane fractions of *E. coli* were always negative.

The biological activity of the recombinant antigen was determined in an *in vitro* antibody-dependent cellular cytotoxicity (ADCC) assay using normal rat eosinophils and sera[16] from rats immunized either with the 25K fusion protein or a solubilized membrane fraction of *E. coli*. In particular samples taken at day 16 showed a high percentage (80%) of cytoxicity towards schistosomula. Heat-inactivation or immunoglobulin E (IgE) depletion on a goat anti-IgE affinity column reduced the killing efficiency of these sera to 35%, indicating that the cytoxicity was mainly due to IgE antibodies. The IgE antibody response to P28 was further confirmed by positive passive cutaneous anaphylaxis (PCA) reactions. Addition of a source of complement factors (guinea pig serum), which may have been inactivated during heat treatment, did not restore the levels of cytotoxicity to the original value. Finally, control rats injected with *E. coli* membrane fractions of comparable composition but lacking the cII fusion protein, showed no significant cytotoxicity in the ADCC assays. These encouraging results led us to attempt a challenge with live cercariae on a series of animals vaccinated with the 25K fusion protein produced in *E. coli*. Both a permissive (golden hamster) and semi-permissive (Fischer rats) host were chosen for this study.

Animals were immunized as described before by two injections with 50 μg of the fusion protein. Six weeks after the second injection, rats and hamsters were infected percutaneously with 1,000 and 250 cercariae respectively. Rats were bled weekly after challenge infection and antisera were used to immunoprecipitate metabolically-labelled proteins of *S. mansoni* adult worms. The sera of infected control rats (Fig. 5, lanes 1 and 2) were not able to immunoprecipitate any antigens of the parasite (Fig. 5, lanes 1 and 2), but animals vaccinated with the recombinant fusion protein showed a strong antibody response against the 28K protein (Fig. 5, lanes 3, 4, 5). The evaluation of parasite burden in rats was performed 21 days after infection, just before the natural parasite rejection, and 30 days after the challenge for the hamster population. The results for both rat and hamster (Fig. 5) show a significant reduction in the number of parasites recovered from those populations which had been immunized with the fusion protein containing the major epitopes of this particular 28K adult worm antigen.

With the next series of immunization experiments we attempted to induce a comparable cell-mediated humoral response in monkeys (baboons). This cytotoxicity could be analysed *in vitro* by incubating the sera in the presence of purified human eosinophils. The sera from two baboons, vaccinated with two injections of either 100 μg or 200 μg of 25K M_r fusion protein, in the presence of aluminium hydroxide, induced

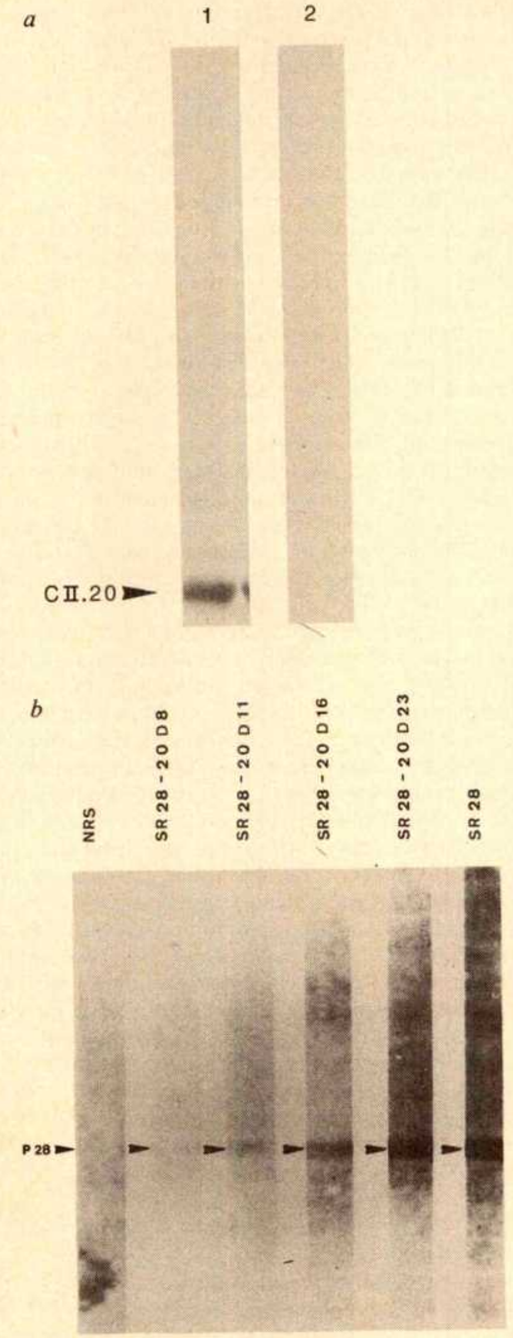

Fig. 3 *a*, Western blotting analysis[19] of the 25K fusion protein. The fusion protein is strongly recognized by an anti-P28 rat serum obtained by immunizing animals with the P28 antigen purified from a crude extract of adult worm (lane 1). A preparation of *E. coli* membrane protein components was used as control (lane 2). CII.20 (arrowed), position of the 25K fusion protein containing 172 amino acids (20K) of the C-terminal region of the 28K antigen and a portion of the *cII* gene. *b*, Kinetic analysis of the humoral response after immunizing rats with the recombinant 28K protein. Western blotting and immunoenzymatic reaction were used to follow the appearance of the antibody response against the 28K protein. Arrow, expected position of P28 antigen. We restricted this study to the IgG/IgM isotypes as markers of immunization of the vaccinated animal immune system. Recognition by immunized rat sera of the native 28K *S. mansoni* antigen occurred at day 11 after the second injection of recombinant antigen, and increased until day 23. SR28-20 represents rat serum against the CII.20 fusion protein obtained 8, 11, 16 and 23 days (D8-D23) after the second injection. SR28, rat serum directed against the native 28K antigen; NRS, normal rat serum.

Methods. Western blots were performed as described[15]. SDS-PAGE-fractionated native 28K antigen (~1 μg) was loaded on each lane of a 13% polyacrylamide slab gel and then transferred to nitrocellulose membrane in 20% methanol, 5 mM Tris-HCl, *p*H 6.8, 75 mM Glycine, for 2 h at 50 mA. Membranes were then saturated with 3% bovine serum albumin (BSA) in PBS and incubated with sera from vaccinated animals obtained 8, 11, 16 and 23 days after the second injection of the 25K fusion protein synthesized in *E. coli.* Rat serum directed against the native 28K antigen (SR28) was used as positive control, and normal rat serum (NRS) as negative control.

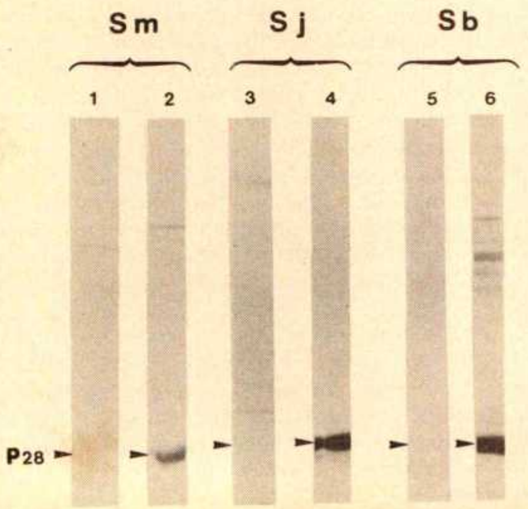

Fig. 4 Identification of epitopes commonly presented by the 28K fusion protein in extracts of related schistosome species. Adult worm homogenates (5 μg) from *S. mansoni* (Sm), *S. bovis* (Sb) and *S. japonicum* (Sj) were fractionated on 13% polyacrylamide slab gels, under denaturing and reducing conditions and electrophoretically transferred to nitrocellulose sheets. Lanes 1, 3, 5, sera from control rats immunized with *E. coli* lysate; lanes 2, 4, 6, sera from rats immunized with the fusion protein. Using SR28-20 D23 serum (Fig. 3), it was shown that the epitopes can equally be detected in these three species of schistosomes and are shared by a protein with M_r 28K. Recently, we have also identified this particular antigen in a crude homogenate from *S. haematobium* adult worm (data not shown). Arrowheads, position of 28K antigen.

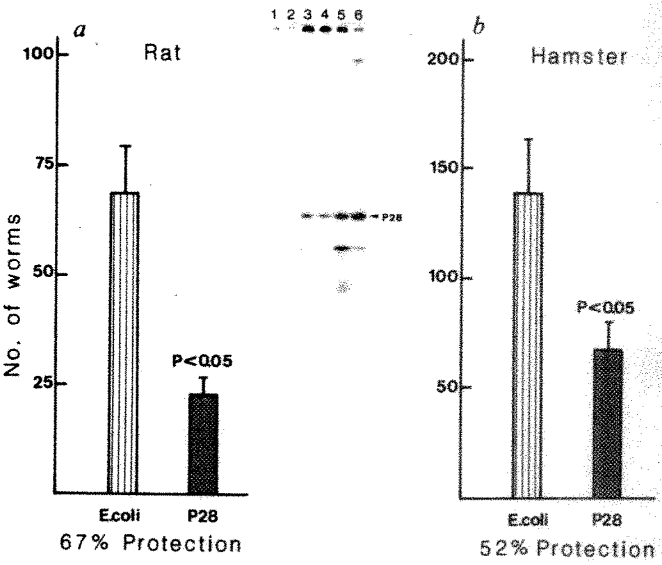

Fig. 5 Vaccination experiments using the 25K fusion protein in rat (*a*) and hamster (*b*). Bars show number of parasites recovered from animals 21 days (rat) or 30 days (hamster) after challenge with cercariae: membrane fractions of *E. coli* (lined bars) were used as controls for the 28K fusion protein (dotted bars); *P* is the probability that the difference is due to chance alone. Inset in *a*, immunoprecipitation of rat serum. After immunization, the antibody response was allowed to return to levels at which no IgG/IgM specific antibodies were detected in the vaccinated rat sera (lane 2) compared to a normal rat serum (lane 1). A challenge infection was performed to exposing rats and hamsters percutaneously to 1,000 and 250 cercariae respectively. The evolution of the post-infection antibody response was analysed. Eight days after the challenge, IgG response directed towards the P28 antigen increased again and remained stable until the day of perfusion as analysed by immunoprecipitation of metabolically ^{35}S-methionine-labelled adult worm proteins (lane 1, normal rat sera; lane 2, infection day; lane 3, day 8 after infection; lane 4, day 15; lane 5, day 21; lane 6, anti-native 28K rat sera). In *b*, the parasite population recovered from hamsters was reduced by 52% ($P < 0.05$).

high levels of eosinophil-dependent cytotoxicity (34.6 ± 14.2% and 95.3 ± 5.6% respectively). Heat-inactivation of these sera reduced the activity to 50% or less. These preliminary results indicated that in monkeys, as in the rat model, both IgG and IgE anaphylactic isotypes are induced by the 28K recombinant antigen. Under the same experimental conditions, naturally-infected baboon serum induced 48.5 ± 10.6% killing efficiency. Sera from control baboons, immunized with SDS-extracted membrane fractions of *E. coli*, induced no significant killing activity of the human eosinophils (<10%). These results correlate with those obtained *in vitro* in the rat and are a basis for subsequent vaccination experiments in primates using more significant numbers of animals.

Conclusions

This paper reports the first successful cloning and expression of a protective antigen present in extracts of adult worms of the parasite *Schistosoma mansoni*. The approach based on screening λgt11 cDNA libraries with specific antibodies proved to be very successful. The structure of this 28K protein is confirmed by partial protein sequence data on the trypsin-treated native antigen, indicating that this protein is indeed a major component of the M_r 28K gel fraction, used as antigen in previous experimental vaccination studies. The cDNA sequence reveals a primary structure of 211 amino acids corresponding to a protein of M_r 28K. The protein expressed in *E. coli* has the same electrophoretic mobility as the native antigen, indicating that the molecule is not extensively modified after translation. Computer-assisted analysis of the protein structure shows neither repetitive elements nor significant similarity with protein sequence data from other parasites.

Injection of a recombinant fusion protein, containing 172 amino acids of the C-terminal part of this 28K antigen, induces a strongly cytotoxic humoral response in rats, hamsters and baboons, composed of both IgE and IgG antibody classes. The first isotype was demonstrated to be a major factor responsible for the significant levels of eosinophil dependent cytotoxicity against schistosomula in vaccinated rats. This observation, although not excluding the function of IgG antibodies, supports the critical role of IgE response in the development of acquired protective immunity against infection by *S. mansoni* or other helminth parasites in general[17].

In conclusion, we report the cloning and expression of a protective antigen which induces high level of antibody-mediated cytotoxicity in rodents or monkeys and in particular immunizes rats and hamsters against experimental infection with live cercariae. Experiments in progress will further test the protective properties of this antigen, which can then be considered as the basis for a vaccine protecting both humans and animals against schistosomiasis.

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Clathrin light chains contain brain-specific insertion sequences and a region of homology with intermediate filaments

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The primary structures of four bovine clathrin light chains have been determined. Light chains LC_a and LC_b are homologous proteins encoded by different genes. In the brain the messenger RNA from these genes undergoes differential splicing to yield proteins having centrally inserted brain-specific sequences. A potentially α-helical region of the clathrin light chains shows homology with intermediate filament proteins.

CLATHRIN is the major protein of the polyhedral coat of coated pits and vesicles[1]. These membrane organelles are involved in various pathways of intracellular transport of which receptor-mediated endocytosis of macromolecules provides well-characterized examples[2,3]. In contrast the role of clathrin-coated organelles in exocytic pathways of secretion is less well defined[4-6].

During receptor-mediated endocytosis clathrin is assembled and disassembled in a regulated fashion[2,7]. Clathrin coats are formed from trimeric molecules called triskelions consisting of three heavy chains of relative molecular mass 180,000 (M_r 180K) and three light chains of M_r 30–40K[8,9]. Triskelions have a characteristic three-armed structure in which each arm consists of one heavy chain and one light chain. There is growing evidence that the heavy chain plays a structural role and that the light chains, located in the triskelion vertex[10,11] are regulatory elements in clathrin function. For example, the light chains provide binding sites for calmodulin[12,13] and an ATP-dependent uncoating protein that disassembles clathrin baskets[14]. In addition there are at least four distinct forms of clathrin light chain that may serve to introduce diversity into the structure and function of coated vesicles in mammalian tissues. Each cell has two distinct light chains, designated LC_a and LC_b. In brain both light chains are of higher apparent M_r than those isolated from other tissues[15-18]. In this paper we describe the structural and genetic basis for the differences between these four forms of clathrin light chains. The accompanying paper[19] localizes different functions in two structurally distinct regions of the light chains.

Primary structure of clathrin light chains

Partial protein sequences were determined for LC_a and LC_b isolated from bovine brain and adrenal gland, a convenient source of non-brain clathrin[18]. Coated vesicles or clathrin triskelions were purified as described by Pearse and Robinson[20], boiled and then centrifuged to give a supernatant that contains clathrin light chains as major components[12,18]. These were further purified by DEAE-cellulose chromatography[21] or by C3 reverse phase (RP) HPLC. Amino-acid sequence analysis of LC_a and LC_b from both tissues indicated that the amino termini of the mature proteins were blocked. Light chains were then cleaved by chemical and enzymatic methods. Peptides produced were purified on C3 and C18 RP-HPLC columns[18] and their amino-acid sequences determined on a gas-phase sequencer. A total of 153 amino-acid residues for brain LC_a, 168 for brain LC_b, 75 for adrenal LC_a and 67 for adrenal LC_b were obtained. From overlapping peptides and the apparent homology between all four light chains, we were able to align almost all of these protein sequences (Fig. 2).

Five oligonucleotides were designed from the protein sequences and used to screen λgt10 complementary DNA libraries made from bovine brain and from a bovine lymphocyte cell line (BL-3). This cell line was a convenient source of messenger RNA for the non-brain light chains[22]. Previous comparison showed that light chains from lymphocyte, adrenal, liver and other 'non-brain' tissues were indistinguishable[16], indicating that lymphocyte cDNAs could be identified with oligonucleotides derived from adrenal protein sequence. The initial screen of 2.5×10^5 recombinants from both libraries was made with one oligonucleotide specific for LC_b and another specific for LC_a. Positive clones were then rescreened with all five oligonucleotides and only those clones hybridizing with two or more were selected for further study. The oligonucleotide screen yielded cDNA clones for lymphocyte LC_a and for lymphocyte and brain LC_b. No clones corresponding to brain LC_a were obtained although one clone corresponding to the non-brain form of LC_a was obtained from the brain library. This was not unexpected as brain contains significant amounts of non-brain light chains[18]. On rescreening the brain cDNA library with the lymphocyte cDNA clone, twenty more clones were obtained. Characterization of three clones revealed one that coded for brain LC_a.

The largest clones that code for each of the four light chains have inserts of 1.1–1.2 kilobases (kb), corresponding to the size of the mRNA as determined by Northern blot analysis. A single, major band of mRNA for each light chain was observed, which was slightly larger in brain than in adrenal gland or the lymphocyte cell line (data not shown).

Southern blot analysis[23] revealed distinct patterns of hybridization with the cDNA probes for LC_a and LC_b (data not shown). This showed that LC_a and LC_b are encoded by distinct genes. For LC_b the patterns of hybridization with digests using eight different restriction enzymes were simple and consistent with a single LC_b gene or multiple closely linked genes. For LC_a the patterns were complex and the results do not allow us to estimate the number of LC_a genes or their patterns of genetic linkage. However the sequence analysis described below provides no evidence for more than one LC_a or LC_b gene.

The nucleotide sequences of the four full-length cDNA clones are shown in Fig. 2*a–d*. The protein sequences predicted from the cDNA sequences are entirely consistent with those obtained by direct analysis (Fig. 2). In particular it should be noted that

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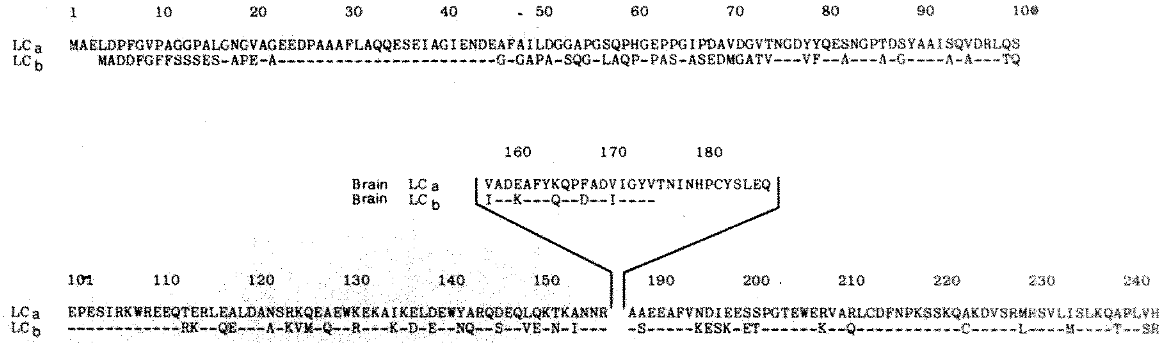

```
         1        10        20        30        40      - 50        60        70        80        90       100
LCa  MAELDPFGVPAGGPALGNGVAGEEDPAAAFLAQQESEIAGIENDEAFAILDGGAPGSQPHGEPPGIPDAVDGVTNGDYYQESNGPTDSYAAISQVDRLQS
LCb     MADDFGFFSSSES-APE-A-----------------------G-GAPA-SQG-LAQP-PAS-ASEDMGATV---VF--A---A-G----A-A---TQ

                  160       170       180
Brain LCa   VADEAFYKQPFADVIGYVTNINHPCYSLEQ
Brain LCb   I--K---Q--D--I----

       101      110       120       130       140       150           190       200       210       220       230       240
LCa  EPESIRKWREEQTERLEALDANSRKQEAEWKEKAIKELDEWYARQDEQLQKTKANNR  AAEEAFVNDIEESSPGTEWERVARLCDFNPKSSKQAKDVSRMKSVLISLKQAPLVH
LCb  ------------RK--QE---A-KVM-Q--R---K-D-E--NQ--S--VE-N-I---  -S-----KESK-ET------K--Q-----------C-----L----M----T--SR
```

Fig. 1 A comparison of the amino-acid sequences of the four clathrin light chains. Numbering refers to the brain LC_a sequence. Amino-acid identities are denoted by a dash, differences are as indicated. The brain-specific inserts are projected. At position 14 in the lymphocyte LC_a sequence an H residue was found, differing from the P residue shown in brain LC_a. This probably results from allelic polymorphism (see text).

the protein sequences derived from adrenal light chains agree completely with cDNA sequences from the lymphocyte cell line.

The cDNA clones encompass the entire coding regions for all four clathrin light chains as judged by the following criteria: (1) all peptide sequences previously obtained are accounted for by the cDNA sequences; (2) the amino-acid compositions predicted from the cDNA sequences agree completely with experimentally determined compositions; (3) for lymphocyte LC_b a stop codon in-frame with the coding sequence is found 136 base pairs (bp) upstream from the initiator methionine; and (4) the cDNA clones and the mRNA are of comparable size. This was important to establish because the predicted M_rs of all the light chains (non-brain LC_b, 23.1K; non-brain LC_a, 23.3K; brain LC_b, 25.1K and brain LC_a, 26.7K) are 30% less than those determined by gel electrophoresis[16]. The anomalous electrophoretic behaviour was found to be a general property of the light chains and their fragments and probably reflects reduced SDS binding.

Differential splicing of brain light-chain mRNA

When the sequences of the four clathrin light chains are aligned (Fig. 1) the structural basis for their polymorphism becomes clear. Comparison of the non-brain forms of LC_a and LC_b shows they have distinct but homologous sequences. Non-brain LC_a is three amino acids longer than non-brain LC_b, correlating with its lower mobility on SDS-gel electrophoresis. There are amino-acid substitutions throughout the non-brain light-chain sequence consistent with LC_a and LC_b sharing a limited number of tryptic peptides[17,18]. In contrast the brain forms of each light chain are identical to their non-brain counterparts except for the presence of inserted sequences at homologous positions about two-thirds of the way from the amino terminus to the carboxy terminus. This result is totally consistent with the similarity in the peptide maps of light chains obtained from brain and adrenal gland[18]. The 18-residue insertion in brain LC_b is homologous to the amino-terminal portion of the 30-residue insertion in brain LC_a. The presence of these inserted sequences explains why brain light chains have lower mobility on SDS gels than the non-brain forms. The increased size of the brain-specific insert in LC_a in addition to the extra three residues at the amino terminus accounts for the lower mobility of brain LC_a compared with brain LC_b.

The unusual pattern of amino-acid sequence identity between the brain and non-brain forms of each light chain is reflected in the nucleotide sequences of their corresponding mRNAs. Except for two point substitutions (discussed below) there is no divergence in the 5′ and 3′ untranslated regions or in the homologous segments of the coding regions. The critical difference is the insertion in the mRNAs encoding the brain forms of either a 54-bp (LC_b) or 90-bp (LC_a) segment encoding the brain-specific sequences.

Even when two non-allelic genes encode identical polypeptides, such as the two human α-globin genes, the nucleotide sequence is conserved in the coding regions but diverges significantly in the 3′ untranslated regions[24]. The total conservation of flanking sequences between the brain and non-brain light chain mRNAs thus indicates that both are initially transcribed from the same genomic sequence.

In addition to the 90-bp insertion sequence there are two single base differences between the cDNA sequences for brain and lymphocyte LC_a. One is a C-to-A substitution that produces a change from histidine to proline at position 14 in the coding region. The other is a T-to-C substitution in the 3′ untranslated region (Fig. 2*a*,*b*). These observations might appear to be inconsistent with the interpretation that LC_a mRNAs in these two tissues derive from a single gene. However, these substitutions probably represent allelic differences between the individual cows from which the brain and lymphocyte libraries were made. It is also possible that they arose from errors introduced by reverse transcriptase in the construction of the libraries[25].

If mRNAs for brain and non-brain forms of clathrin light chains are the products of single LC_a and LC_b genes, then their differences must result from differential splicing of the initial RNA transcripts. The additional sequences in the brain forms would then result from the expression of exons that are spliced out in other tissues. Differences in the sequences of the cDNA clones obtained from brain and the lymphocyte cell line are also seen at the poly(A) addition site. The lymphocyte cDNA for LC_b has an additional two guanine residues (CTTGGAA compared to CTTAA) and the lymphocyte cDNA for LC_a has an additional AAAT sequence (AAGAAATAA compared to AAGAA) (Fig. 2*a*-*d*). This indicates that the processing of light-chain mRNA follows quite a different pathway in brain compared to other tissues.

Two patterns of tissue-specific splicing have been found to generate alternative forms of fibronectin[26]: exon skipping, in which entire exons are spliced out of the mRNA, and exon subdivision, in which alternative splice acceptor sites in an exon are used. For LC_a the latter mechanism might be employed because of the presence of a canonical splice acceptor site at the 3′ end of the brain-specific insertion sequence. In the case of LC_b no such site is present indicating that an exon skipping mechanism operates. A short region of sequence homology exists between the first eight residues of the brain-specific insert and the first eight residues on the carboxy-terminal side of the insert (Fig. 1). This may result from the existence of regulatory sequences that are involved in the splicing reactions or from the requirements for a particular feature of protein structure at this point in the sequence.

Conserved and variable regions

Alignment of the amino-acid sequence for LC_a and LC_b (Fig. 1) reveals 60% identity indicating that the LC_a and LC_b genes diverged from a common ancestor that underwent duplication. However, the light-chain sequences have not been uniformly *a*

```
     TTGCCTCTGATAGCAGGCGCAAGCACAGCGGCCGCTGCTGGAGTTGGTGTTTGCTGGTCGGTTGGTGTTTCTCTGAAAGTCGGTGCCGTC
   1 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 90

     GAACTTCCCGCCATGGCTGAGTTAGATCCGTTCGGCGTCCCCGCTGGCGGTCCGCCCTGGGGAACGGAGTGGCCGGCGAAGAGGATCCT
  91 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 180
                  M  A  E  L  D  P  F  G  V  P  A  G  G  P  A  L  G  N  G  V  A  G  E  E  D  P   -

     GCCGCGGCCTTCTTGGCGCAGCAGGAGAGCGAGATTGCGGGCATAGAGAACGACGAGGCCTTCGCCATCCTGGACGGCGGCGCCCCCGGA
 181 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 270
      A  A  A  F  L  A  Q  Q  E  S  E  I  A  G  I  E  N  D  E  A  F  A  I  L  D  G  G  A  P  G   -

     TCCCAGCCTCACGGCGAGCCGCCGGGGATTCCAGATGCTGTTGATGGAGTGACGAATGGAGACTACTACCAGGAGAGTAATGGGCCGACA
 271 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 360
      S  Q  P  H  G  E  P  P  G  I  P  D  A  V  D  G  V  T  N  G  D  Y  Y  Q  E  S  N  G  P  T   -

     GACAGTTATGCAGCTATTTCACAAGTGGATCGATTGCAGTCAGAGCCTGAAAGTATCCGTAAATGGAGAGAAGAGCAAACGGAACGCTTG
 361 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 450
      D  S  Y  A  A  I  S  Q  V  D  R  L  Q  S  E  P  E  S  I  R  K  W  R  E  E  Q  T  E  R  L   -

     GAAGCCCTTGATGCCAATTCTCGGAAGCAGGAAGCGGAGTGGAAAGAAAAAGCAATAAAGGAGCTGGACGAGTGGTATGCGAGGCAGGAT
 451 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 540
      E  A  L  D  A  N  S  R  K  Q  E  A  E  W  K  E  K  A  I  K  E  L  D  E  W  Y  A  R  Q  D   -

     GAGCAGCTACAGAAGACAAAAGCCAACAACAGGGTGGCAGATGAAGCTTTCTACAAACAACCCTTCGCTGACGTGATTGGTTATGTCACA
 541 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 630
      E  Q  L  Q  K  T  K  A  N  N  R  V  A  D  E  A  F  Y  K  Q  P  F  A  D  V  I  G  Y  V  T   -

     AACATAAACCATCCTTGCTACAGCCTAGAACAGGCAGCAGAAGAAGCCTTTGTAAATGACATTGAGGAGTCGTCCCCAGGCACTGAGTGG
 631 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 720
      N  I  N  H  P  C  Y  S  L  E  Q  A  A  E  E  A  F  V  N  D  I  E  E  S  S  P  G  T  E  W   -

     GAACGGGTGGCCCGGCTGTGTGACTTTAACCCCAAGTCCAGCAAGCAGGCCAAAGATGTCTCCCGCATGCGTTCTGTCCTCATCTCCCTC
 721 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 810
      E  R  V  A  R  L  C  D  F  N  P  K  S  S  K  Q  A  K  D  V  S  R  M  R  S  V  L  I  S  L   -

     AAGCAGGCCCCCCTGGTGCACTGAAGAGCCGCCCTGTGGAAACACTACATCTGCAATATCTTAATCCTACTCAGTGAAGCTCTTCACGGT
 811 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 900
      K  Q  A  P  L  V  H  *

     AATTGGATTAATTATGTTGAGTTCTTTTGGACCAAACCTTTTGTCTTTAGAGTTGATCATTGTTTGTGATTGCATGTTTCCTTCAACTGT
 901 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 990

     GTTCTCCCTGGCATTCAAAGAGGAGGGGAGGTGGCGGCAGAGGAAGGGAGGGAGGCCTCCCAACGGCAGCCTCAACCTATGCTTTTGTGC
 991 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 1080

     ATTATTCTGAGAATAAATTTCTGTTCCAAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
1081 ---------+---------+---------+---------+---------+---------+---------+-------- 1158
```

b

```
     CAAGCACAGCGGCCGCTGCTGGAGTTGGTGTTTGCTGGTCGGTTGGTGTTTCTCTGAAAGTCGGTGCCGTCGAACTTCCCGCCATGGCTG
   1 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 90
                                                                                         M  A  E -

     AGTTAGATCCGTTCGGCGTCCCCGCTGGCGGTCACGCCCTGGGGAACGGAGTGGCCGGCGAAGAGGATCCTGCCGCGGCCTTCTTGGCGC
  91 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 180
        L  D  P  F  G  V  P  A  G  G  H  A  L  G  N  G  V  A  G  E  E  D  P  A  A  A  F  L  A  Q -

     AGCAGGAGAGCGAGATTGCGGGCATAGAGAACGACGAGGCCTTCGCCATCCTGGACGGCGGCGCCCCCGGATCCCAGCCTCACGGCGAGC
 181 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 270
        Q  E  S  E  I  A  G  I  E  N  D  E  A  F  A  I  L  D  G  G  A  P  G  S  Q  P  H  G  E  P -

     CGCCGGGGATTCCAGATGCTGTTGATGGAGTGACGAATGGAGACTACTACCAGGAGAGTAATGGGCCGACAGACAGTTATGCAGCTATTT
 271 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 360
        P  G  I  P  D  A  V  D  G  V  T  N  G  D  Y  Y  Q  E  S  N  G  P  T  D  S  Y  A  A  I  S -

     CACAAGTGGATCGATTGCAGTCAGAGCCTGAAAGTATCCGTAAATGGAGAGAAGAGCAAACGGAACGCTTGGAAGCCCTTGATGCCAATT
 361 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 450
        Q  V  D  R  L  Q  S  E  P  E  S  I  R  K  W  R  E  E  Q  T  E  R  L  E  A  L  D  A  N  S -

     CTCGGAAGCAGGAAGCGGAGTGGAAAGAAAAAGCAATAAAGGAGCTGGACGAGTGGTATGCGAGGCAGGATGAGCAGCTACAGAAGACAA
 451 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 540
        R  K  Q  E  A  E  W  K  E  K  A  I  K  E  L  D  E  W  Y  A  R  Q  D  E  Q  L  Q  K  T  K -

     AAGCCAACAACAGGGCAGCAGAAGAAGCCTTTGTAAATGACATTGAGGAGTCGTCCCCAGGCACTGAGTGGGAACGGGTGGCCCGGCTGT
 541 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 630
        A  N  N  R  A  A  E  E  A  F  V  N  D  I  E  E  S  S  P  G  T  E  W  E  R  V  A  R  L  C -

     GTGACTTTAACCCCAAGTCCAGCAAGCAGGCCAAAGATGTCTCCCGCATGCGTTCTGTCCTCATCTCCCTCAAGCAGGCCCCCCTGGTGC
 631 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 720
        D  F  N  P  K  S  S  K  Q  A  K  D  V  S  R  M  R  S  V  L  I  S  L  K  Q  A  P  L  V  H -

     ACTGAAGAGCCGCCCTGTGGAAACACTACATCTGCAATATCTTAATCCTACTCAGTGAAGCTCTTCACGGTAATTGGATTAATTATGTTG
 721 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 810
        *

     AGTTCTTTTGGACCAAACCTCTTGTCTTTAGAGTTGATCATTGTTTGTGATTGCATGTTTCCTTCAACTGTGTTCTCCCTGGCATTCAAA
 811 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 900

     GAGGAGGGGAGGTGGCGGCAGAGGAAGGGAGGGAGGCCTCCCAACGGCAGCCTCAACCTATGCTTTTGTGCATTATTCTGAGAATAAATT
 901 ---------+---------+---------+---------+---------+---------+---------+---------+---------+ 990

     TCTGTTCCAAGAAATAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 991 ---------+---------+---------+---------+---------+------ 1046
```

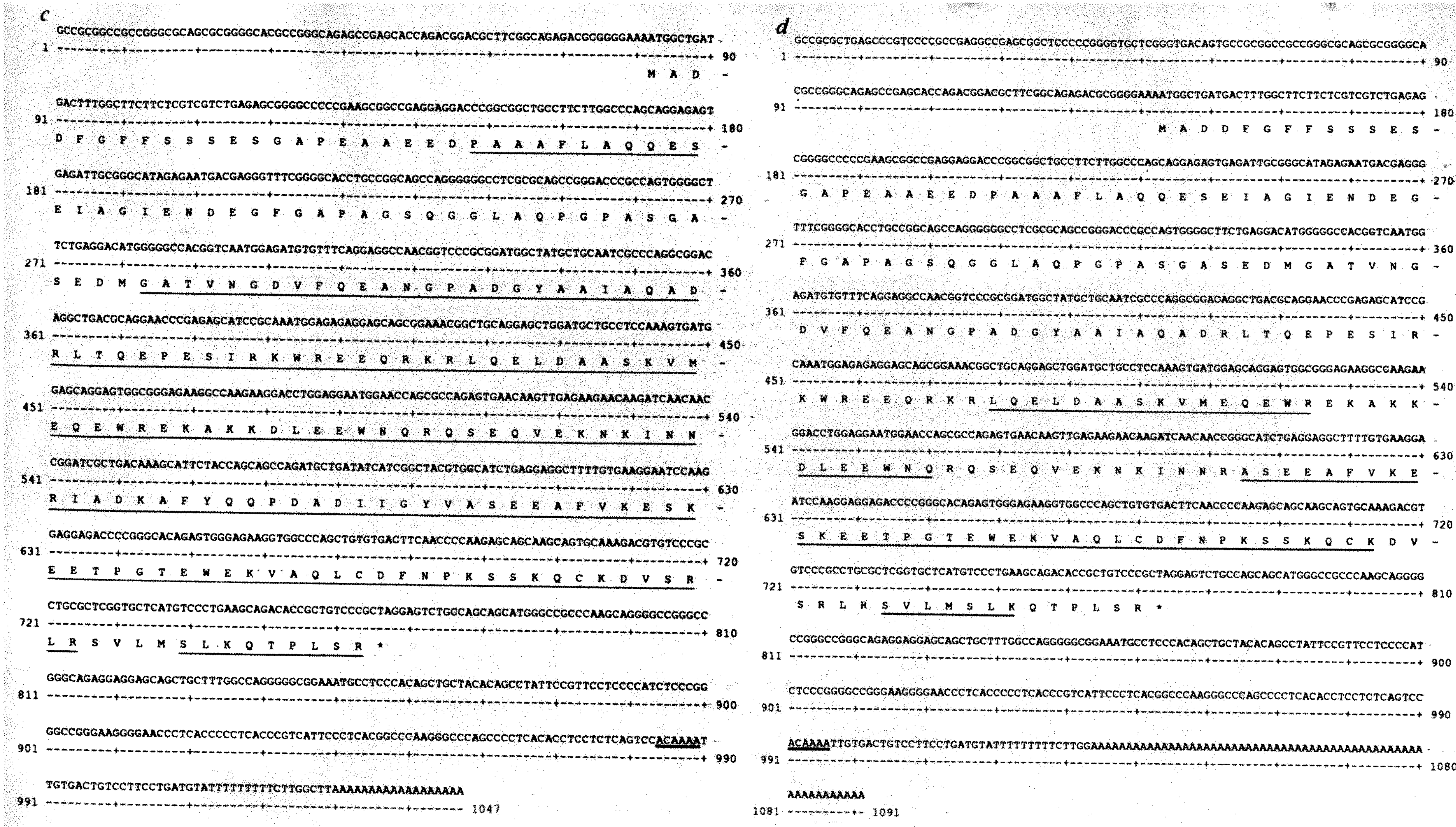

Fig. 2 The nucleotide and predicted amino-acid sequences of *a*, brain LC$_a$; *b*, lymphocyte LC$_a$; *c*, brain LC$_b$ and *d*, lymphocyte LC$_b$. The two nucleotide differences between brain and non-brain LC$_a$ outside the tissue-specific insert are underlined. Putative polyadenylation signals are double underlined[38]. The underlined amino-acid sequences were independently confirmed by means of peptide sequencing.

Methods. Full length cDNA clones were subcloned in both orientations into the *Eco*RI site of M13mp18. Single-stranded DNA was prepared and sequenced by the dideoxy method using [α-^{35}S]dATP[39]. The complete sequence on both strands was obtained by subcloning and sequencing restriction enzyme fragments and by the use of oligonucleotides specific for internal sequences[40]. Sequences were assembled and analysed using computer programs of Staden[41] and the University of Wisconsin[42].

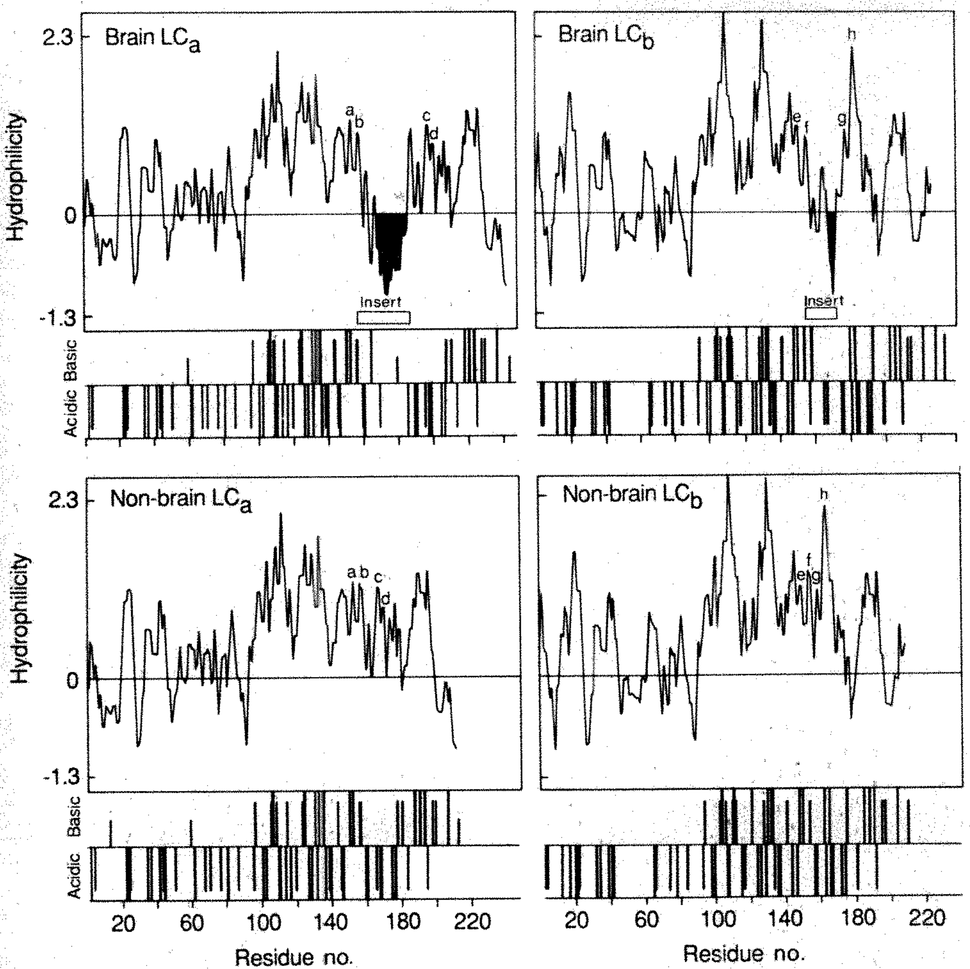

Fig. 3 Hydrophilicity plot and charge distribution for clathrin light chains. The plots record the average hydrophilicity along the sequences over a window of nine residues[30]. Hydrophilic and hydrophobic residues are in the upper and lower part of the frame respectively. Peaks that flank the brain-specific insertion sequences and are shared by the brain and non-brain forms of LC_a and LC_b are marked a, b, c, d and e, f, g, h respectively. Below each panel is the distribution of charged residues with basic residues above the line with histidines represented by the shortest lines, lysines by the longest and arginine by lines of intermediate length; and acidic residues below the line, with aspartic acid as the shorter and glutamic acid as the longer lines.

conserved. A striking feature is at the amino-terminal end of the proteins. An uninterrupted block of 23 conserved amino acids is flanked by two stretches of roughly 20 amino acids which represent the most divergent portions of the molecules. In the amino-terminal diversity region of LC_b there are four serine residues in a stretch of five amino acids which are not found in LC_a. One or more of these residues may be the site of phosphorylation that only occurs in LC_b[27-29]. In contrast to the amino-terminal region, the distribution of conserved residues is more uniform in the carboxy-terminal 60% of the polypeptides.

A clear differentiation in the distribution of charged and hydrophobic amino acids between the amino-terminal 40% and carboxy-terminal 60% of the molecule is also apparent (Fig. 3). Although there are no large hydrophobic segments in either portion of the molecule, the charged residues are largely confined to the carboxy-terminal region. More strikingly, the overall acidic character of the light chains reflects the fact that virtually all the charged residues in the amino-terminal 40% of the molecule are aspartic and glutamic acids. These observations emphasize the chemically distinct nature of the two regions of the light chains which is correlated with the antigenic and functional differences described in the accompanying paper[19].

A comparison of the hydrophilicity plots[30] for both LC_a and LC_b shows that brain-specific sequences introduce a prominent hydrophobic peak that is not found in the lymphocyte forms (Fig. 3). In coated vesicles the brain-specific sequences are exposed and these hydrophobic elements may therefore interact with molecules or organelles of the brain cytoplasm[19].

All species of higher vertebrates examined show two classes of clathrin light chains that presumably correspond to LC_a and LC_b. In contrast, yeast expresses a single light chain[5]. The persistence of two light-chain classes in the evolution of higher eukaryotes, their expression in all tissues and their significant differences in structure all point to the conclusions that the two light chains are functionally differentiated and that both are required for clathrin function.

Homology with intermediate filaments

A computer search of the NBRF (National Biomedical Research Foundation) protein databank with the program FASTP[31] revealed an interesting similarity between the central region of the clathrin light chain and the α-helical domain of various intermediate filament proteins, particularly the cytokeratins[32]. This similarity (Fig. 4) was judged to be of statistical significance by the program RDF[31]. Further support for the significance of the homology is the observation that residues which are highly conserved between intermediate filament proteins tend to be those that are also shared with the clathrin light chains (residues marked with a triangle in Fig. 4).

Homology between clathrin light chains and intermediate filament proteins has two major implications. First, the amino-acid sequence similarities suggest that the structures of the homlogous segments may be similar. Specifically, it is known that the segment of the intermediate filament proteins marked 'coil' in Fig. 4 forms α-helices which are involved in the coiled coil structure characteristic of the intermediate filaments[33]. Predictive analysis of this portion of the light-chain sequences by two independent methods indicates a high helix-forming potential for this part of the protein[34,35]. However, it is unlikely that this helix would be involved in a coiled coil structure such as is found in intermediate filaments. Sequences forming a coiled coil have a pattern of hydrophobic amino acids that are spaced alternatively at 3 and 4 residues[36]. These positions in the intermediate filament proteins are designated with a filled circle in

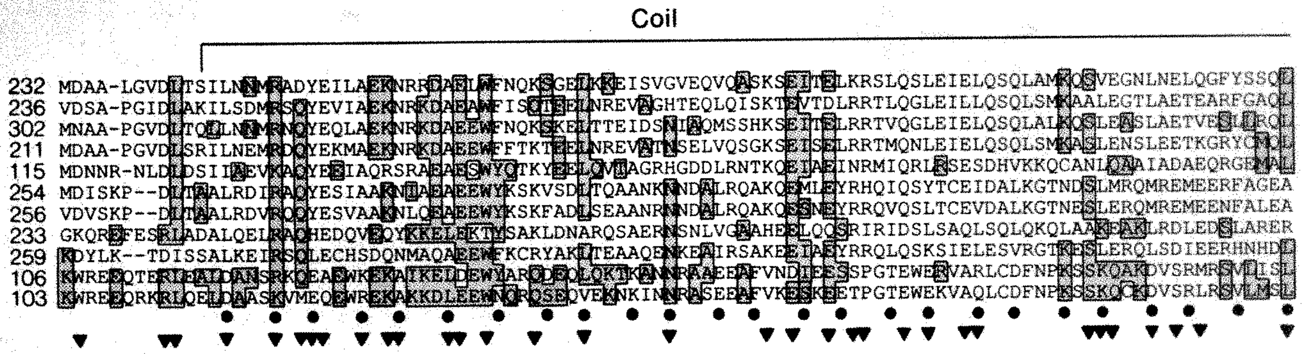

Fig. 4 Homology between the central region of each non-brain clathrin light chain (residues 107–236) and the α-helical domain of intermediate filaments. Amino-acid identities are boxed. ▼, Positions in the intermediate filaments where amino acids have been conserved in seven out of nine proteins; ●, positions in the intermediate filaments of hydrophobic residues comprising the 3/4 periodicity which contributes to the formation of coiled coil structures[36]. The sequences are of the following proteins: *a*, *Xenopus laevis* embryonal cytokeratin[43]; *b*, bovine type I cytokeratin[43]; *c*, mouse type I cytokeratin[44]; *d*, human type I cytokeratin[33]; *e*, human type II cytokeratin[33]; *f*, chicken desmin[33]; g, hamster vimentin[33]; *h*, human nuclear lamin[45]; *i*, porcine neurofilament[33]; *j*, clathrin LC_a; *k*, clathrin LC_b.

Fig. 4. In the light-chain sequences there is little conservation of hydrophobicity at these positions. The prediction that the light chains will not form coiled coils is consistent with Ungewickell's observations that free light chains do not form oligomers[21]. In the accompanying paper, we show that the region of homology with intermediate filaments is involved in interaction with the clathrin heavy chain[19].

The similarity shown in Fig. 4 suggests that the intermediate filament proteins and the clathrin light chains are evolutionarily related to each other. The finding that homology is restricted to only one segment of each protein is not uncommon and is believed to result from the shuffling of exons by recombination[37]. Current analysis of the structure of the light-chain genes will reveal whether the region of homology with intermediate filaments is encoded by a separate exon. If so, then the introduction of this exon during the evolution of the light-chain genes may have provided the structure necessary for binding to the heavy chain.

Conclusions

These studies have determined the structural basis for the four polypeptide forms of clathrin light chain so far identified. None of the differences is trivial and the results strongly indicate that considerable functional diversification of the clathrin light chains has occurred in eukaryotic evolution. Thus we believe that all four light chains will be shown to have both common and unique functions. The specialization between LC_a and LC_b may be understood by comparison of the function of clathrin in yeast and in mammalian cells. Explaining the remarkable differences between the brain and non-brain forms of the clathrin light chains will clearly require further definition of the functions of coated vesicles in different tissues. In the accompanying paper we relate the structure of the clathrin light chains to their interactions in coated vesicle function[19].

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Interstellar diamonds in meteorites

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Primitive meteorites contain up to 400 p.p.m. of a very fine-grained type of carbon, noncommittally called Cδ[1]. It apparently comes from outside the Solar System, as it carries isotopically anomalous krypton and xenon ('Xe–HL' or 'CCFXe', enriched twofold in the lightest and heaviest isotopes[2]) and nitrogen ($\delta^{15}N = -330‰$[3]; that is, depleted in ^{15}N by −330‰ relative to atmospheric nitrogen), although the carbon itself is within the terrestrial range ($\delta^{13}C = -38‰$[1]). Expanding on a preliminary report[4], we now present evidence that part or all of Cδ is diamond—not shock-produced but primary, formed by stellar condensation as a metastable phase. It appears that interstellar dust contains diamond.

Thinking that Cδ might be the C_{60} cluster 'buckminsterfullerene'[5], we isolated a new sample from the Allende C3V chondrite, aiming for maximum purity rather than maximum yield. After dissolving the major minerals in HF–HCl, we generated a colloid from the remaining 0.5% residue, leaving behind coarse-grained spinel and chromite[2,6]. The major gas-poor part (Cγ) of the colloidal carbon was removed by oxidation with 16 M and 21 M HNO_3 (16 h, 70 °C) and concentrated $HClO_4$ (2 h, 140 °C). Surprisingly, the residue (Allende CH, 109 p.p.m.) was not black but light tan. As it contained some silicate contaminants, we reprocessed it with HCl and HF, obtaining Allende CJ (89 p.p.m.) of unchanged colour.

On the scanning electron microscope (SEM) Allende CJ showed no elements above oxygen (Mg, Al and Si <2.5 wt% if evenly dispersed or ≪1% if in grains of >0.5 μm). X-ray diffraction showed only three very broad, diffuse lines, which to our surprise matched the three principal lines of diamond. On the transmission electron microscope (TEM), the grains turned out to be typically 50 Å in size (Fig. 1). Electron diffraction (Fig. 2) confirmed that most grains were diamond, as shown by the agreement of the observed spacings with accepted values (shown in parentheses): 2.06 (2.06), 1.26 (1.26), 1.07 (1.075), 0.893 (0.8916), 0.817 (0.8182), 0.73 (0.728), 0.68 (0.686) Å. Elemental analysis by analytical electron microscopy showed a small amount of Cl (contamination or surface-bound Cl from HCl?) as well as Cr and Fe (submicrometre-sized ferrichromite[7]). Examination by high-resolution mass spectrometry at 70–550 °C showed only small amounts of volatile material, probably contamination. Peaks appeared up to at least $m/e = 733$, but none of the more abundant ones corresponded to C_{60} or other C_n ions.

To check whether such diamonds are present in other meteorites containing Xe–HL, we isolated similar residues from Murchison (C2), Murray (C2) and Indarch (EH4), using $HClO_4$ at higher temperature (190–206 °C) to remove the more reactive forms of carbon. Residues Murchison GL1 (white, 225 p.p.m.) and Indarch CF (dark grey, 36 p.p.m.) both showed diamond by electron diffraction. Murray A2C (white, 250 p.p.m.) was not checked by electron diffraction but showed only minor Al and Si on the SEM.

From the TEM work it appears that some amorphous, low-atomic-number material is present in addition to diamond, perhaps radiation-damaged diamond or unrelated extraneous material. (As we note later, Xe–HL seems to have been trapped by ion implantation, and presumably was accompanied by large amounts of other ions.) This is supported by density-gradient centrifugation, showing that Murchison GL1 has an effective bulk density of 2.22–2.33 g cm^{-3}, well below the density of pure diamond (3.51 g cm^{-3}). This material is not graphite or amorphous carbon, else the sample would not be pure white. But its amount must be less than implied by the low effective density, as the particles sink not singly but as aggregates, containing much interstitial liquid immobilized in angstrom-sized voids.

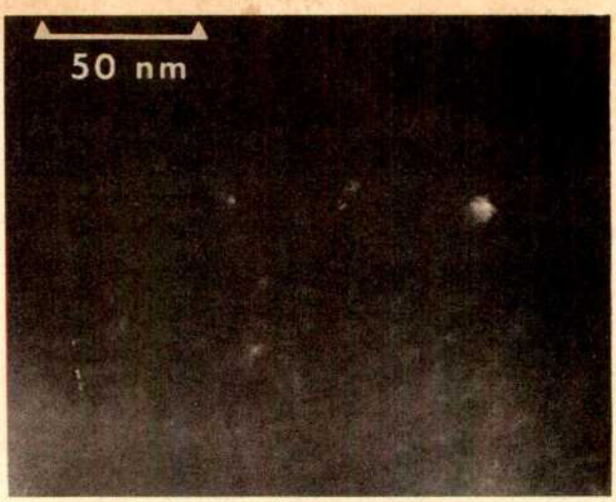

Fig. 1 Dark-field transmission electron micrograph of Allende CJ, in which bright areas are diamond grains diffracting electrons into one part of the innermost ring of the diffraction pattern shown in Fig. 2. As most grains do not have the proper orientation, the true abundance of diamond is perhaps five times higher than indicated by the bright areas in the picture.

Curiously the infrared spectrum strongly resembles that of soot, with peaks at 2.93, 3.17, 5.59, 6.13 and 7.14 μm. For soot, these features are attributed to the reaction of surface atoms with H and O, and presumably such surface alteration also occurs for fine-grained diamond. Approximately 9% of the atoms in 50-Å diamond are at the surface.

On heating, Cδ showed a surface graphitization behaviour consistent with that of diamond. A sample wrapped in platinum foil did not change when heated in air (19 days at 165 °C, 3 days at 280 °C, 1 day at 380 °C), but blackened visibly at ~10^{-2} torr (1 day at 380 °C). A sample heated in a Pyrex tube in high vacuum darkened only slightly (1 h at 100–550 °C, 3 min at 550 °C). Diamond behaves similarly, darkening at low O_2 pressures but not in high vacuum or at high O_2 pressures (where any graphite formed is rapidly oxidized).

Given that Cδ is mainly diamond, is the diamond the actual carrier of the isotope anomalies? We cannot get absolute proof, as our samples are not pure diamond; the best we can do is establish correlations between the anomalies and the diamond content of the samples.

The Xe–HL content is indicated by ^{136}Xe–HL (Table 1). It is generally highest in the white samples, although black Allende B1B is close and white Murray A2C (which contained some extraneous phases) is lower. Apparently Xe–HL stays with the diamond when other phases are destroyed. The highest values for Allende and Murchison are similar ($17\text{–}19 \times 10^{-8}$ cm^3 g^{-1}), as expected if both meteorites contained the same kind of Cδ.

The correlation with other anomalies is more tenuous, as no data are available yet on the new samples. Earlier measurements by stepped combustion show that light N, light C, and Xe–HL are all released in the same temperature range of 500–600 °C[1,3,8], and hence may be located in the same phase. However, this correlation must be checked on diamond-enriched samples. (The combustion temperature of terrestrial diamond under identical conditions is ~250 °C higher[9], but that is a trivial consequence of the coarser grain size. At an activation energy of 60 kcal mol^{-1}, the combustion rate per unit area is 4.8×10^3 times higher at 800 °C than at 550 °C, but this difference would be exactly offset by a 70-fold difference in grain size, that is, 3,500 Å rather than 50 Å).

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Diamonds have been previously found in some meteorites (most ureilites and two iron meteorites), but they apparently formed by shock, in terrestrial or asteroidal impacts[10,11]. It seems unlikely that the Cδ diamonds originated in this way. First, although all four meteorites in which these diamonds have been found are somewhat brecciated (Allende least so), none shows the strong shock effects, high post-shock temperatures, or both, that characterize other diamond-bearing meteorites. Second, all the Cδ-bearing meteorites also contain 10^3–10^4 p.p.m. of graphitic or amorphous carbon that has no Xe–HL or light N and is isotopically heavier ($\delta^{13}C = -15‰$, compared with $-38‰$ for Cδ[1,12]). If the diamonds were made from graphite grains in the meteorite parent bodies by shock, why did the shock (in three or four different parent bodies) singlemindedly transform only the Xe–HL-bearing grains, while ignoring the more abundant, gas-poor carbon grains? Third, all four meteorites have essentially similar Cδ, with comparable gas contents and isotopic compositions (Table 1). If the diamonds were shock-produced, we would expect greater variations, depending on the proportions of the two types of carbon converted to diamond and on post-shock diffusion losses. Ureilites, for example, show a ~100-fold variation in gas concentration[13].

By default, it seems necessary to invoke an extra-solar origin for the diamond. But diamond has not been seriously considered a possible constituent of the cosmos outside the Solar System, as no astronomical object except planets has the right pressure-temperature conditions for its formation as a stable phase (40–400 kbar, 1,200–4,000 K), and any diamonds formed in planets could not get out because of the high escape velocity. (Landau[14] has pointed out that diamond of a steep size distribution would give a good match to the interstellar extinction curve, but he did not consider the origin of the diamonds.) Although shocks are common in the cosmos, they too, do not give the combination of high pressure and moderate temperature required for formation of diamond. The only feasible mechanism would seem to be metastable formation of diamond from a hot gas at low pressures, as recently demonstrated in the laboratory[15]. Let us see how this mechanism can be incorporated in a scheme that accounts for Xe–HL.

Xe–HL apparently formed in a supernova[16] and (along with other noble gases, H and N) was trapped in carbon grains by ion implantation[17]. The high relative velocities (~10^3 km s^{-1}) required for the latter process imply that the grains predate the supernova, and presumably formed in the gas shell ejected by the pre-supernova during its red giant or planetary nebula stage some 10^2–10^4 yr earlier[18]. Carbon is the first solid expected to condense from a carbon-rich gas at equilibrium[19], and although graphite is the stable phase, diamond, being metastable by only ~2 kJ mol^{-1}, could form instead. Indeed, such preferential formation of diamond has been observed in the laboratory[15]. The processes studied so far involve decomposition of CH_4 or organic compounds in the presence of large amounts of hydrogen, either by ordinary pyrolysis or by plasma reactions. The mechanism is not yet known, but seems to involve CH_3 radicals and H atoms. Although CH_3 is not an abundant radical in red giants, other radicals or molecules, such as CH, C_2H and C_2H_2, may be able to serve in its place. Whatever the mechanism, it now seems necessary to add diamond to the list of interstellar grain constituents. We review here some of the prospects opened up by this work.

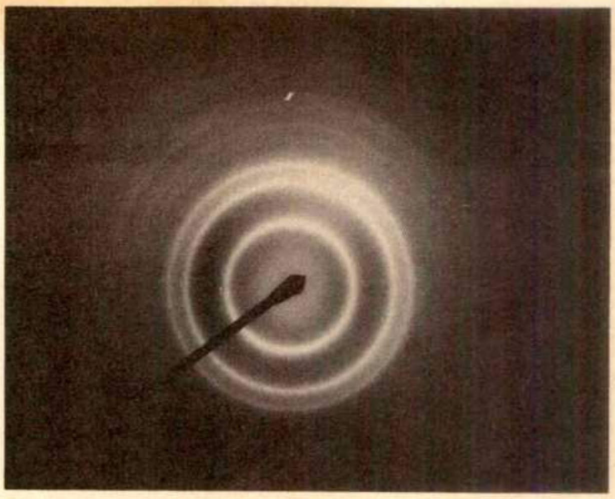

Fig. 2 Selected-area electron diffraction pattern from Allende CJ. This is a typical ring powder pattern, with *d*-values matching those of diamond.

First, diamond should be looked for in space wherever graphite is thought to be present: circumstellar, interstellar and interplanetary dust (including dust from comets). Perhaps the abundance of interstellar graphite has been overestimated; Nuth[20] has pointed out that most of the carbon in primitive meteorites is a complex organic polymer, not graphite, and now that Cδ has turned out to be diamond, the balance is even more lopsided: graphite constitutes ≤2,000 p.p.m., compared to 100–400 p.p.m. diamond and $(2\text{–}3) \times 10^4$ p.p.m. organic carbon. But meteorites may not be representative of interstellar matter. Several authors have argued that a single, massive supernova exploded near the forming Solar System, injecting short-lived

Table 1 Anomalous Xe in diamond-rich meteorite separates

Sample		Class	Mass fraction* p.p.m.	Gas content 10^{-8} cm^2 g^{-1} ^{132}Xe	^{136}Xe–HL†	$\frac{^{136}Xe}{^{132}Xe}$	References
Allende	CJ‡	C3V	89	30±6	19±4	0.642±3	This paper
Allende	CH‡	C3V	109	24±2	15±1	0.649±3	This paper
Allende	B1B	C3V	390	25±3	16±2	0.644±5	3
Allende	CC	CeV	180	21±2	14±1	0.653±23	3
Allende	B1E	C3V	180	16±2	10±1	0.636±8	This paper
Allende	CG	C3V	250	12±1	8±1	0.652±3	6
Murchison	GL1‡	C2	225	37±7§	17±3	0.550±2§	This paper
Murchison	2C10f	C2	780	18±2§	8±1	0.537±2§	23
Murray	A2C‡	C2	250	28±2§	12±1	0.535±2§	This paper
Indarch	CF	EH4	36	9±2	5±1	0.593±4	This paper

* Mass fraction of separate relative to bulk meteorite. Values for different samples are not directly comparable. In the earlier samples they are high due to extraneous phases and in the later, highly purified, samples they are low due to mechanical losses.

† ^{136}Xe–HL is the calculated excess ^{136}Xe in the sample, relative to planetary Xe with $^{136}Xe/^{132}Xe = 0.32$.

‡ White; all other samples are black (except Indarch CF, which is dark grey) owing to the presence of graphitic carbon.

§ Excluding an isotopically normal Xe component released at 800 °C, before the release of Xe–HL (≥1,000 °C). It occurs only in C2 chondrites[23]. Its carrier, Cζ is carbonaceous but totally uncharacterized.

radionuclides as well as excess amounts of the stable isotopes ^{12}C, ^{16}O and ^{20}Ne (refs 21, 22). If the Solar System is indeed enriched in supernova products, then Cδ and Xe-HL (whether from the same or another supernova) may be atypically high.

Second, if diamond is detected in circumstellar dust, its formation conditions (as inferred from the atmosphere of the central star) should be determined and duplicated, insofar as possible, in the laboratory. Possibly nature makes diamond more efficiently than even the best laboratory synthesis discovered so far.

Finally, meteoritic diamond should be isotopically analysed for all detectable elements. If the xenon was trapped by ion implantation from a supernova, then it ought to be accompanied by the remainder of the periodic table. Diamond, owing to its extreme durability, may be an informative record of stellar nucleosynthesis and chemistry and of the prehistory of the Solar System.

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Metastable carbon in two chondritic porous interplanetary dust particles

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An understanding of carbonaceous matter in primitive extraterrestrial materials is an essential component of studies on dust evolution in the interstellar medium and the early history of the Solar System. We have suggested previously that a record of graphitization is preserved in chondritic porous (CP) aggregates and carbonaceous chondrites[1,2] and that the detailed mineralogy of CP aggregates can place boundary conditions on the nature of both physical and chemical processes which occurred at the time of their formation[2,3]. Here, we report further analytical electron microscope (AEM) studies on carbonaceous material in two CP aggregates which suggest that a record of hydrocarbon carbonization may also be preserved in these materials. This suggestion is based upon the presence of well-ordered carbon-2H (lonsdaleite) in CP aggregates W7029*A and W7010*A2. This carbon is a metastable phase resulting from hydrous pyrolysis below 300–350 °C and may be a precursor to poorly graphitized carbons (PGCs) in primitive extraterrestrial materials[2].

Chondritic porous aggregates are samples of unmelted micrometeorites that survived atmospheric entry without appreciable effects of thermal metamorphism due to pulse-heating[3-5]. Broadly based classification studies and chemical analyses of stratospheric dust collections show that fluffy aggregates of small-sized grains (<100 nm) with a chondritic bulk composition are commonly of extraterrestrial origin[6,7]. The bulk carbon content of CP aggregates can range up to 5 wt% in carbon-rich CP aggregates[6] and possibly includes hydrocarbon compounds[8]. For this study we selected two CP aggregates from the Johnson Space Center (JSC) Cosmic Dust Collection. Detailed information on collection, curation and processing procedures at the JSC Curatorial Facility are given by Rietmeijer and Mackinnon[3] and references therein.

Chondritic porous aggregate W7029*A is a carbon-rich, uncontaminated sample of primitive extraterrestrial material[3]. During preparation for AEM study parts of this aggregate broke apart on to the holey carbon substrate. Thus, individual particles from the aggregate were suitably dispersed and accessible to study[2-4]. In CP aggregate W7029*A about 44% of all individual particles are carbonaceous material which occurs as (1) poorly graphitized carbon[2]; (2) amorphous carbon[9] and (3) a well-ordered carbon phase. The remainder of grains in this CP aggregate are predominantly low-temperature minerals[3]. The presence of kaolinite[3] and cubic Bi_2O_3[4] in this CP aggregate suggests that temperatures on atmospheric entry were no higher than ~300 °C. While CP aggregate W7010*A2 also belongs to the carbon-rich group of CP aggregates, its carbonaceous material mainly occurs in its matrix as the embedding medium for ultra fine-grained (1-150 nm) silicates, sulphides and oxides[10]. Only a small portion of individual grains in this aggregate is PGC and a well-ordered carbon phase.

Analyses were performed with a modified JEOL 100CX AEM[9] equipped with a Princeton Gamma Tech (PGT) System IV Energy Dispersive Spectrometer (EDS) operating at an accelerating voltage of 100 kV. All EDS spectra are consistent with the presence of low atomic number ($Z < 11$) elements only. For selected grains we confirmed the carbon chemistry using a JSM-35CF scanning electron microscope fitted with a PGT Windowless EDS (WEDS) operating at an accelerating voltage of 10 kV and by WEDS and electron energy loss spectroscopy (EELS) using a Philips EM 420T operating at 120 kV. The samples are not provided with a conductive surface coating (for example, Au/Pd or C) before analysis. Sample contamination with carbon materials during laboratory handling or in the AEM is negligible[3,11]. Identification of phases is possible through a combination of AEM imaging, selected area electron diffraction (SAED), EDS, WEDS and EELS analyses. The diffraction spacings shown in Table 1 have a relative error of ~1%. For individual particles we obtained SAED data at various sample tilt angles relative to the incident electron beam.

The majority of carbonaceous material in CP aggregate W7029*A is present as clumped masses of thin sheets and as irregular fluffy grains. The morphology and crystallographic properties of material in these occurrences are consistent with poorly graphitized carbon[2]. Thin, amorphous carbon rims on pre-existing minerals similar to those observed in other CP aggregates[12] are also present in CP aggregate W7029*A[4]. In addition, a small fraction (~10%) of carbonaceous material in this CP aggregate occurs as thin (<100 Å) electron transparent sheets which range from 0.2 μm × 0.3 μm to 0.6 μm × 2.0 μm in size (Figs 1 and 2). In general, these sheets have lobate circumferences, but in rare cases show a subhedral outline. Most of this carbonaceous material occurs as single sheets, although stacking of sheets is occasionally observed. When exposed to the electron beam the smooth surfaces of these sheets may become slightly wrinkled, suggesting loss of volatiles. In CP aggregate W7010*A2, the morphology of carbonaceous grains which are not part of the matrix, is similar to the thin sheets in CP aggregate W7029*A. SAED data for each aggregate shown in Table 1 are for the carbon phase in these sheets and are consistent with crystalline carbon-2H, or lonsdaleite. However, the orientation

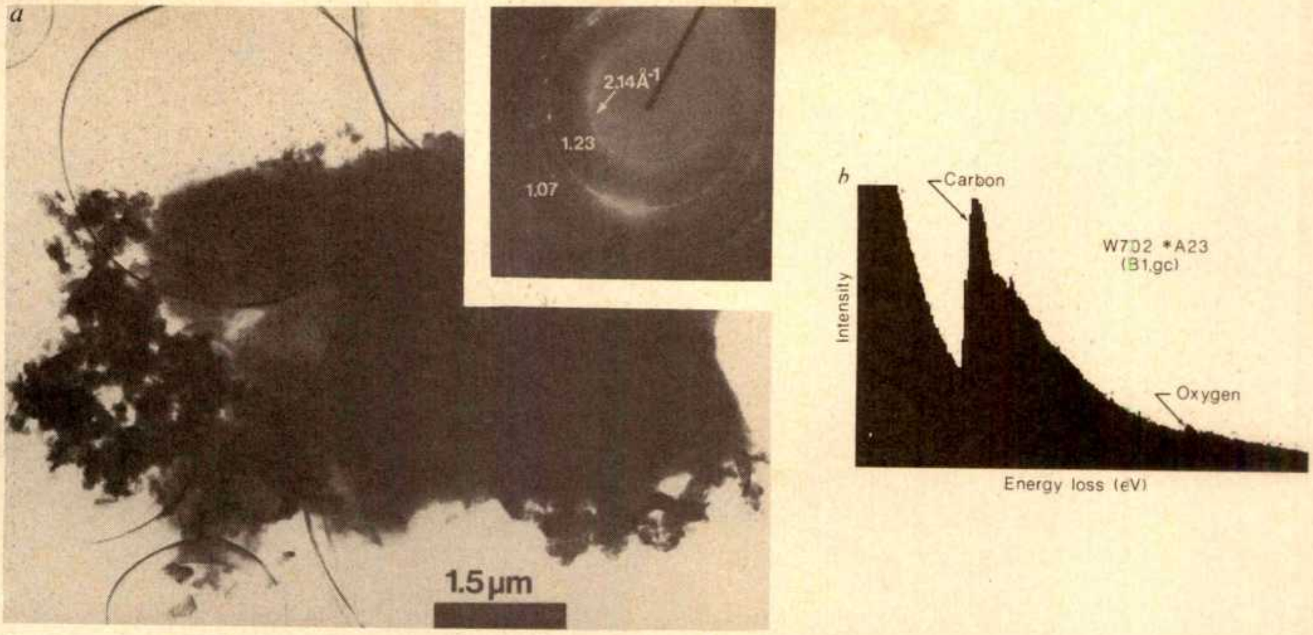

Fig. 1 *a*, Large carbon-2H with regions of poorly graphitized carbon (left-hand side). The SAED pattern (inset) is consistent with carbon-2H. *b*, Electron energy loss spectrum on thin, smooth carbon-2H grain shown in *a*.

of the thin sheets parallel to the sample support film interferes with acquisition of all diffraction spacings typical of carbon-2H. With the exception of two diffraction spacings, all other diffraction spacings for carbon-2H and PGC in the samples overlap. Nevertheless, in conjunction with the known absence of high-Z elements in these sheets and the fact that no known carbon phase mimics these two diffraction spacings[13], we identify the thin sheets as crystalline carbon-2H.

The SAED data (Table 1) show that carbons in CP aggregate W7029*A, and to a lesser extent in CP aggregate W7010*A2, occur mainly in two distinct forms as carbon-2H and PGC. In the PGCs the d_{002} basal spacing is typically at 3.50±0.03 Å. However, the majority of PGC particles in both CP aggregates also show carbon-2H interplanar spacings and this suggests that these carbon particles are a mixture of PGC and carbon-2H. For comparison, interplanar spacings for carbon residues from carbonaceous chondrites are also listed in Table 1. Carbons in these meteorites are primarily PGC[14].

We have previously suggested that PGC in CP aggregate W7029*A and in carbonaceous chondrites results from graphitization of a carbon phase[2]. The graphite reflection, d_{101} (2.10 Å) in PGC from CP aggregates W7029*A and W7010*A2 is more diffuse and less defined when compared to the same reflection from PGC in carbonaceous chondrites. This reflection is in fact a composite of the (101) and (100) reflections (d = 2.03 Å and 2.13 Å) and is a sensitive indicator of the onset of graphitization[15]. The value of the (002) spacing in PGC is a measure of the degree of order, or the extent of graphitization, for PGC[2,15]. The above observations suggest that graphitization in carbonaceous chondrite PGC is better developed than that observed for PGC in CP aggregates.

In order to understand the occurrence of carbon-2H in CP

Table 1 Interplanar spacings (Å) for carbon sheets from 11 and 9 individual grains in CP aggregates W7029*A and W7010*A2, respectively, compared with interplanar spacings (Å) for carbon-2H and poorly graphitized carbons in CP aggregate W7029*A and selected carbonaceous chondrites

hkil	W7029*A	W7010*A2	Carbon-2H	*hkil*	W7029*A	Allende CV3	Cold Bokkeveld C2	Orgueil CI
				002	3.50 (3.47–3.53)	3.47 (3.39–3.51)	3.6 (3.4–3.9)	3.8 (3.5–4.1)
$10\bar{1}0$	2.185±0.015	2.17	2.18		2.18±0.03	—	—	—
				100	2.09	2.10	2.10	2.09
0002	—	—	2.06					
$10\bar{1}1$	—	—	1.93					
				004	1.75±0.03	1.73	—	—
					1.635	—	—	—
$10\bar{1}2$	—	—	1.50					
$11\bar{2}0$	1.26±0.01	1.265	1.26	—	1.265±0.015	—	—	—
	1.195±0.01	1.19		110	1.19±0.02	1.22	1.21	1.21
$10\bar{1}3$	—	—	1.17	006	1.16	1.16	—	—
$11\bar{2}2$	1.085±0.015	1.09	1.075	200	1.09±0.02	1.06	1.05	—
				106	1.01	—	—	—
					0.92	—	—	—
					0.87	—	—	—
22i0	0.81±0.02	—	—	210	0.82±0.02	0.83	—	—
	0.72±0.015	—	—	300	0.725±0.015	0.71	—	—

Data for carbon 2H from ref. 19, for W7029*A from ref. 2 and for carbonaceous chondrites from ref. 14.

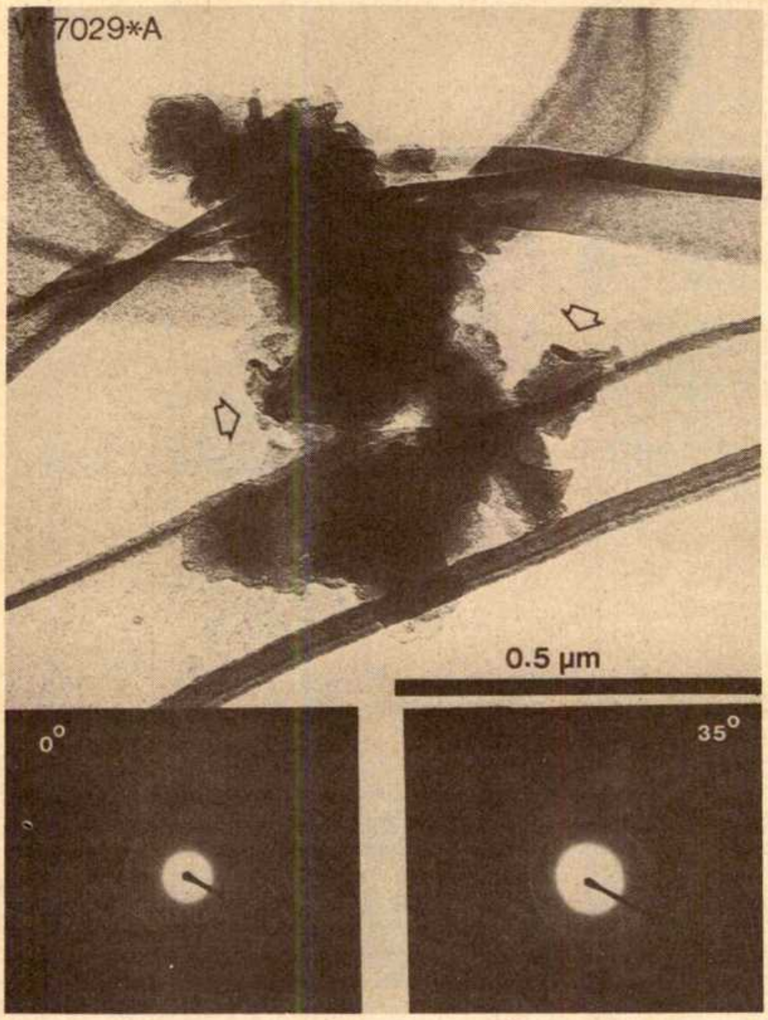

Fig. 2 Carbon-2H in CP aggregate W7029*A showing small areas of electron beam damage (arrowed). SAED patterns are shown for two different tilt orientations relative to the incident electron beam and are typical for carbon-2H.

aggregates, we refer to literature on low-temperature alterations of (hydro-) carbon compounds because the presence of layer silicates and PGC in these CP aggregates indicate aqueous activity at temperatures <315 °C[2,3]. As a likely reaction path may be hydrous pyrolysis of hydrocarbons, we would like to draw attention to materials and conditions prevalent during hydrous pyrolysis of kerogens in natural terrestrial environments[16,17] and to those that are represented in CP aggregate mineralogy[2,3]. In addition, we discuss the formation of carbon-2H as a low-temperature metastable phase because other processes for the formation of stable carbon-2H in an interstellar, or early Solar System, environment are not readily invoked for primitive extraterrestrial materials such as CP aggregates. For example, carbon-2H is a stable high-temperature and high-pressure polymorph of carbon that can form from pure graphite at temperatures >1,000 °C and static pressures >130 kbar[18]. Carbon-2H may also form via intense shock metamorphism of pure graphite[19,20]. However, solar flare track[5] and noble gas[21] data show that CP aggregates are not part of larger (for example, meteoroid size) bodies during Solar System transit. Thus, intense shock events through impact with meteoroids, or larger-sized bodies, are unlikely formation mechanisms for carbon-2H in CP aggregates. In addition, parent bodies for CP aggregates are probably porous and of low density (for example, comet-like[22]) and would not provide an environment suitable for high-temperature and/or high-pressure transformations. It is conceivable that carbon-2H resulting from a parent body brecciation process may have been introduced as a foreign component to the CP aggregates. Two lines of evidence argue against introduction of carbon-2H that formed elsewhere prior to CP aggregate formation: (1) lack of evidence for shock metamorphism in silicates (for example, olivines) in the CP aggregates[23] which would be expected from a brecciation process[24] and (2) the association of carbon-2H and PGC in CP aggregate W7029*A. Thus, we explore carbon-2H formation by a process other than shock metamorphism, such as *in situ* formation by hydrous pyrolysis[3]. This process in terrestrial environments produces metastable carbon-2H[25].

Catalytically activated hydrous pyrolysis of hydrocarbons, particularly kerogens, is an important process for the diagenesis of terrestrial hydrocarbon deposits at temperatures <350 °C[16,17,26]. Kerogen is thermodynamically unstable and in a terrestrial environment, with increasing time and temperature, will decompose into successively more stable phases such as graphite, CO_2, CH_4, and N_2. This irreversible process may proceed along complex pathways with a variety of intermediate phases[17,26,27]. Also, the pyrolysis temperature of the reactions is more important than the effect of total pressure or the presence of a catalyst[17,26]. An important fraction of carbonaceous material in micrometeorites may occur as hydrocarbons as suggested by infrared and ultraviolet spectra of circum- and interstellar dust, the physical properties of carbons in CP aggregates and optical properties of carbonaceous material in several CP aggregates[8,23]. As we have shown in previous studies on PGC and layer silicates in CP aggregate W7029*A, the physical environment (for example, pressure and temperature conditions) which characterizes the aggregate compares favourably with terrestrial hydrous pyrolysis[2-4,22]. In addition, layer silicates similar to that which catalytically activate terrestrial hydrous pyrolysis[16] have been observed in CP aggregates[3,28]. The efficiency of the catalytic process depends, among other parameters, on the possibility of readsorption of pyrolysis products into the surface of catalysts[16]. In this respect, the porous structure of the aggregates may be conducive to exposure of large amounts of surface area to an active aqueous fluid[3,22]. Most importantly, of all possible pyrolysis processes, only hydrous pyrolysis produces the soft, that is well-graphitizable, carbons[26] which, with continued heat-treatment, can transform to PGC[15]. We propose that at the low temperatures required for these primitive parent bodies[22], the kinetics of alteration reactions will dominate this catalytic hydrous pyrolysis mechanism. Thus, for conditions likely to occur after CP aggregate formation (see above) carbon-2H forms as a crystalline metastable product of catalytically activated hydrous pyrolysis of hydrocarbons (following the Ostwald step rule) in primitive extraterrestrial materials. This proposition may even prove tenable for some carbons in many carbonaceous chondrites[29,30]. For example, a variety of layer silicates have been observed in CP aggregates[3,23] and in CI and CM carbonaceous chondrites[31]. Previous observations on carbon phases in both types of primitive extraterrestrial materials[2] as well as the possible conditions of their formation[1,22,29] suggest that low-temperature diagenesis involving catalytic reactions may be a primary mechanism for the formation of the mineralogy observed in many CP aggregates and some components of carbonaceous chondrites. A precise understanding of the complex pathways for the formation of carbonaceous compounds and carbon phases may allow detailed interpretations of isotopic variations (for example changes in D/H ratios, see ref. 27) noted for CP aggregates[32] and carbonaceous chondrites[33].

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A novel observational test of momentum balance in a solar flare

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A unique combination of Solar Maximum Mission X-ray spectra and Sacramento Peak Observatory Hα imaging spectra has enabled us, for the first time, to measure and compare momentum values of upflowing and downflowing plasmas during the impulsive phase of a solar flare. We observed the well-known blue asymmetry of X-ray spectral lines[1], indicative of upflow, in the coronal Ca XIX line. We simultaneously observed the red asymmetry of Hα line profiles, indicative of downflow, in bright Hα kernels. We find that, to within observational uncertainty, the momentum transported by the upflowing X-ray plasma was the same as that of the downflowing Hα material. Of the several physical mechanisms advanced[2] to explain the observed blue asymmetry of X-ray lines, only explosive chromospheric evaporation predicts oppositely directed momenta of equal amplitude.

Impulsive hard X-rays from the small C2 flare were detected by the Solar Maximum Mission (SMM) hard X-ray burst spectrometer from 23:43:42 to 23:44:33 on 30 April 1985. Before this burst the region showed enhanced counts in the O VIII and Mg XI channels of the SMM flat crystal spectrometer (FCS). From the flux ratios we infer a preflare coronal temperature $T_0 \approx 3\times10^6$ K. According to coronal loop equilibrium scaling laws[3,4] and the loop length inferred from the Hα flare images, this corresponds to a preflare pressure of $P_0 \approx 12$ dyn cm^{-2} in the corona and upper chromosphere.

Soon after the peak of soft X-ray emission, at 23:45 UT the FCS was pointed to the brightest feature within its field and a spectroscopic scan of the helium-like Ne IX triplet was obtained. Following the technique of Wolfson *et al.*[5] we derive a coronal electron density of $n_e \approx 8\times10^{10}$ cm^{-3}. The SMM bent crystal spectrometer (BCS) observed the whole flare, both spatially and temporally, but to measure accurately the Doppler shift of the blue component of the Ca XIX spectrum it was necessary to average over the 70 s time period preceding soft X-ray maximum. Figure 1 compares these data to synthetic spectra. From this fit we derive a mean value of the upflow velocity $v_e \approx 290\pm100$ km s^{-1}. From the strength of the Ca XIX line as a whole we derive a total coronal emission measure EM $\approx 3\times10^{48}$ cm^{-3} at soft X-ray maximum.

Hα imaging spectra[6] were obtained using the vacuum tower telescope at the Sacramento Peak Observatory (SPO). Preflare-subtracted Hα line profiles in the brightest Hα-emitting pixel at representative times during the impulsive hard X-ray burst

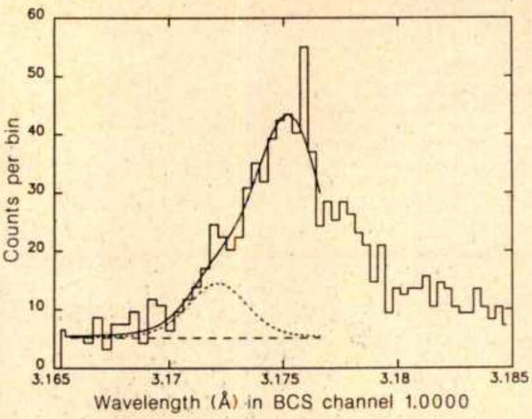

Fig. 1 A comparison of Ca XIX spectra observed with the SMM bent crystal spectrometer and a synthetic spectral fit. The continuous curve is the synthetic fit, which includes the background indicated by the long-dashed line, a symmetric unshifted component, and a blue-shifted component given by the short-dashed curve, implying an upflow velocity of 290 ± 100 km s^{-1}.

are shown in Fig. 2. For the ensuing analysis we have examined all pixels that contribute to the total redshifted region of cross-sectional area $S_c \approx 4 \times 10^{17}$ cm^2. On average, profiles in this region show a peak redshift velocity of ~60 km s^{-1}, which falls by a factor of 2 in about 35 s. For purposes of comparison with the X-ray data we will adopt an average velocity $v_c \approx 30$ km s^{-1} over a characteristic time $\tau_c \approx 70$ s.

Following the method of Ichimoto and Kurokawa[7], we derive the momentum of the coronal plasma by assuming that the total emission measure $\mathrm{EM} = n_e^2 V$ of the soft X-ray emitting plasma of density n_e and volume V is provided by chromospheric evaporation in a timescale τ_e. Thus, conservation of mass yields the relation

$$mn'v_eS_e\tau_e = mn_eV = \frac{m\mathrm{EM}}{n_e} \tag{1}$$

where m, n', v_e and S_e are the mean mass per nucleon, density, velocity, and cross-sectional area of upflowing plasma, and we do not assume that $n' = n_e$. The total momentum that is associated with this material is

$$p_e = mn'v_e^2S_e\tau_e \tag{2}$$

where we have assumed constant v_e during τ_e. By eliminating n', S_e, and τ_e we obtain

$$p_e = \frac{mv_e\mathrm{EM}}{n_e} \tag{3}$$

Substituting parameter values from the previous section, we obtain $p_e \approx 2 \times 10^{21}$ g cm s^{-1}.

Explosive chromospheric evaporation[8] creates a cool downward-moving condensation[9] behind a radiating shock[10]. Its rate of mass accretion per unit area is given by mn_0v_c, where n_0 is the preflare (unshocked) chromospheric density and v_c is the downward condensation velocity. Consequently its momentum after τ_c is approximately

$$p_c = mn_0v_c^2S_c\tau_c \tag{4}$$

Assuming chromospheric-coronal pressure equilibrium before the flare, the inferred preflare pressure $P_0 \approx 12$ dyn cm^{-2} and a typical preflare chromospheric temperature $T_0 \approx 8{,}000$ K imply a preflare density $n_0 \approx 1 \times 10^{13}$ cm^{-3}. We therefore estimate that $p_c \approx 6 \times 10^{21}$ g cm s^{-1}.

The almost equal amplitudes and opposite signs of the momenta of upflowing X-ray and downflowing Hα material have interesting implications for the debate[2] over the physical interpretation of observed impulsive phase X-ray blueshifts. Two of the competing interpretations are not consistent with our observations of this flare. In the magnetohydrodynamic model[2,11] one expects both X-ray and Hα material to be ejected outward, in contradiction to our present observation that they are oppositely directed. In the reconnecting-loop model[2], most of the X-ray emitting material is static, confined by a strong magnetic field to a low-lying high-pressure loop. It is easy to show[1] that the high coronal pressures created impulsively in the low-lying loop will drive extremely fast downflows (~200 km s^{-1}) in the Hα-emitting chromosphere at the footpoints. Such dominance by downflowing Hα-emitting material contradicts our present observation of oppositely directed flows carrying equal amounts of momentum. In the explosive chromospheric evaporation model[8], the sudden creation of a high-pressure region at the footpoint of a coronal loop creates an opposite action and reaction, balancing upward and downward momentum. Of these three models, only the explosive chromospheric evaporation model predicts the observed equal momentum content of upflowing and downflowing material during the impulsive phase.

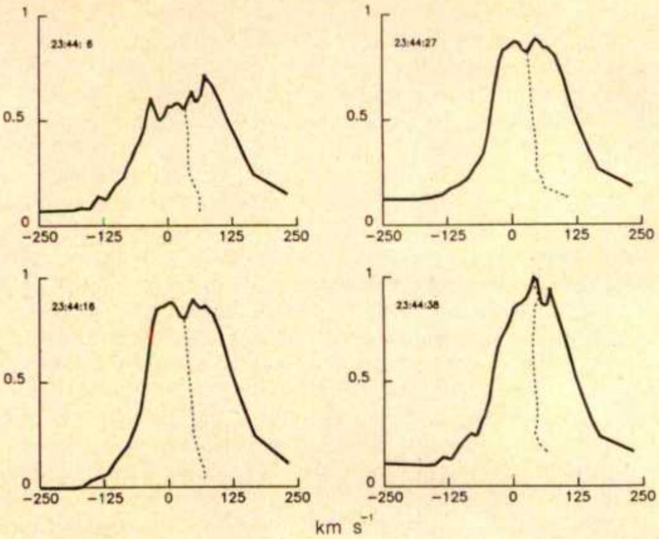

Fig. 2 SPO Hα profiles in the brightest pixel, after subtraction of the preflare profile, showing a pronounced red asymmetry. Dotted lines indicate profile centroids. The shifts of the centroids in the extreme wings imply a peak condensation velocity for this pixel of about 50 km s^{-1}, falling by a factor of 2 on a timescale of about 30 s.

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Mechanism for geomagnetic polarity reversals

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Recent studies of old magnetic observations[1] and the seismic velocity of the lower mantle[2] suggest that at least part of the geomagnetic secular variation is driven by temperature anomalies in the solid mantle[3]. In particular, a patch of flux of opposite sign to that expected for a dipole field occurs beneath southern Africa. It has intensified throughout the twentieth century, by a process that is believed to be flux expulsion associated with a particularly hot part of the lowermost mantle, and is drifting westwards. A similar patch lies beneath South America. Here I show that the present fall in the dipole moment is directly related to the intensification and southward movement of these patches and suggest the fall occasionally leads to polarity reversal. The almost linear increase in the frequency of polarity reversals that has occurred since the Cretaceous quiet interval is attributed to the growth of the hot patch of mantle presently under southern Africa.

A review of palaeointensity measurements[4] has shown a clear fall in the dipole moment throughout the past 2,000 years; there is good evidence for growth in the preceding 2,000 years. Modern data also exhibit this fall in dipole moment: Fig. 1 shows the fall, in the twentieth century, of the Gauss coefficient g_1^0, which is proportional to the dipole moment M

$$M = 4\pi a^3 g_1^0/\mu_0 \qquad (1)$$

where a is the Earth's radius. Clearly, recent secular variation data sample a small section of a long-term process of dipole decay, and we may hope to understand something of the mechanism of dipole decay by studying details of the present secular variation.

The fall in dipole moment has not been steady even in recent times. Figure 1 shows a rapid rate both now and earlier in the century, and a lower rate (15nT yr^{-1}) around 1945. The field model for epoch 1842.5[1] suggests a fall at the lower rate for the second half of the nineteenth century, consistent with the palaeomagnetic results[4].

How can this rapid fall in dipole moment be related to secular variation of the core field? At the core-mantle boundary g_1^0 is related to the radial field by an integral over the core surface

$$g_1^0 = (3c^3/8\pi a^3)\int Z\cos\theta\, dS \qquad (2)$$

where c is the core radius, θ is colatitude and Z is the downward vertical component of the magnetic field. According to equation (2), a plot of $Z\cos\theta$ on the core-mantle boundary, in equal area projection, will indicate those features that contribute most to the dipole moment. Such a plot is shown in Fig. 2 using a field model for epoch 1945 derived by fitting a cubic time dependence through each Gauss coefficient of main field models (see ref. 1 and J. Bloxham, D.G. and A. Jackson, in preparation) for every decade from 1905.5 to 1955.5, 1966.0, 1969.5 and 1980.0. The dipole moment is proportional to the area average of the quantity plotted in Fig. 2. Clearly the main positive contributions come from the four features marked 1-4 called the 'main lobes' in the field by Bloxham and Gubbins[1]. The only significant negative contributions come from features 5 and 6, the 'reverse flux' features, where radial field points in the opposite direction to that expected for a dipole (see Fig. 1 of ref. 1).

The main lobes have not moved substantially throughout historical time, but the reverse flux features have drifted westwards at rates of some tenths of a degree per year. The former may be associated with the main dynamo process[6] whereas the latter appear to form near Indonesia and drift west to intensify strongly beneath southern Africa by expulsion of toroidal flux[1,5]. Flux expulsion is believed to be caused by fluid upwelling associated with a region of hot mantle, a part of the mantle that has low seismic velocity[2]. Mantle anomalies are stationary for long periods of time, and therefore associated drifting reverse flux patches will be a persistent feature of the secular variation throughout the recent geological past.

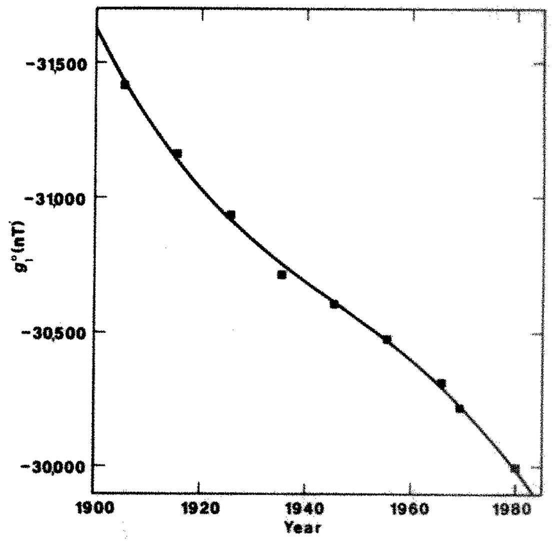

Fig. 1 Changes in the Gauss coefficient g_1^0, which is proportional to the dipole moment, throughout the twentieth century. The points are values from core field models (ref. 1 and J. Bloxham, D.G. and A. Jackson, in preparation) the line is a best-fitting cubic used to calculate the main field and secular variation used in this paper (Figs 2 and 3).

Changes in the dipole moment can be related to secular variation in the core field using the time derivative of equation (2) and a model for the secular variation. A model was obtained by differentiating the cubic fit to the main field models and truncating the result at degree 8. It compares well with other models based on observatory annual means[7] but lacks their detail in the short-timescale features, such as jerks, which I wish to avoid here. Epoch 1945 was chosen to avoid inaccuracies in the cubic fit at the ends of the range.

Figure 3 shows that the fall in dipole moment results almost entirely from reverse flux features in the Southern Hemisphere—secular variation in the Northern Hemisphere (Fig. 3a) is low everywhere. A region of reverse flux can contribute to a change in dipole moment in two ways: either by intensifying *in situ* or by moving southwards into a region of larger cos θ. Feature 6 has intensified whereas feature 5, near South America, has moved south. The positive region of Fig. 3b is due mainly to the westward drift of feature 6.

In AD 1715, the earliest date for which core-field maps are available, feature 5 appeared in the central south Atlantic[1]. Since then it has drifted westwards and increasingly southwards; its westward movement has been 35° of longitude in 265 yr (0.13° yr^{-1}). Feature 6, which is now beneath southern Africa, seems to have formed in the nineteenth century, drifted rapidly westward, and intensified in the early twentieth century. There is some evidence that it broke from an undulation in the magnetic equator beneath Indonesia in the late eighteenth century[1]. The undulations are a persistent feature of the region and Gubbins and Bloxham[1] speculated that they are associated with core-mantle boundary topography, in which case they would be another long-term feature of the secular variation. If this identification is correct then feature 6 has drifted at a much faster rate than feature 5, by some 60° in 200 years (0.3 yr^{-1}); it must eventually catch up with feature 5 because of its faster rate of drift. Its greater speed can be seen in the models for the last

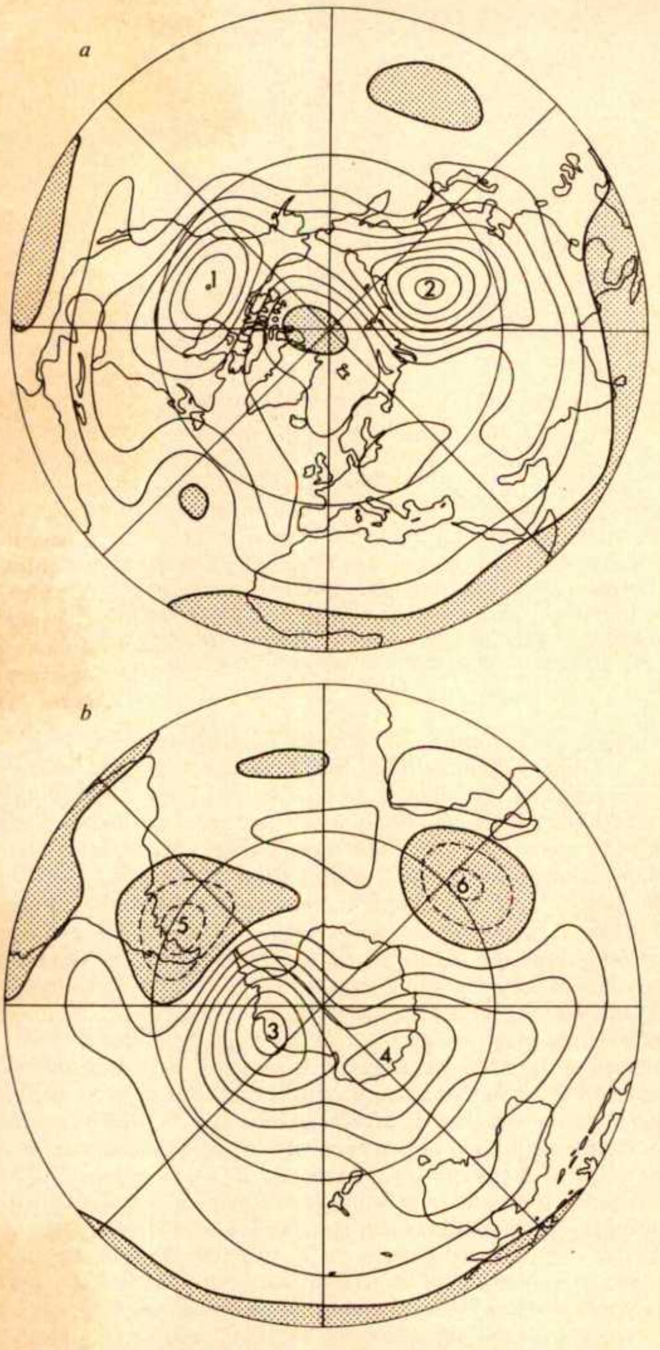

Fig. 2 Magnetic field at the core-mantle boundary for epoch 1945. The component plotted is $Z \cos \theta$, where θ is colatitude, contour interval 100 μT, Lambert equal area projection with pole of the projection at *a*, the North geographic Pole and *b*, the South Pole. The dipole moment is proportional to the integral of this function over the surface. Clearly the main contribution to the dipole moment comes from the four main lobes marked 1-4. The only significant negative contributions come from the two reverse flux features (5,6) in the Southern Hemisphere.

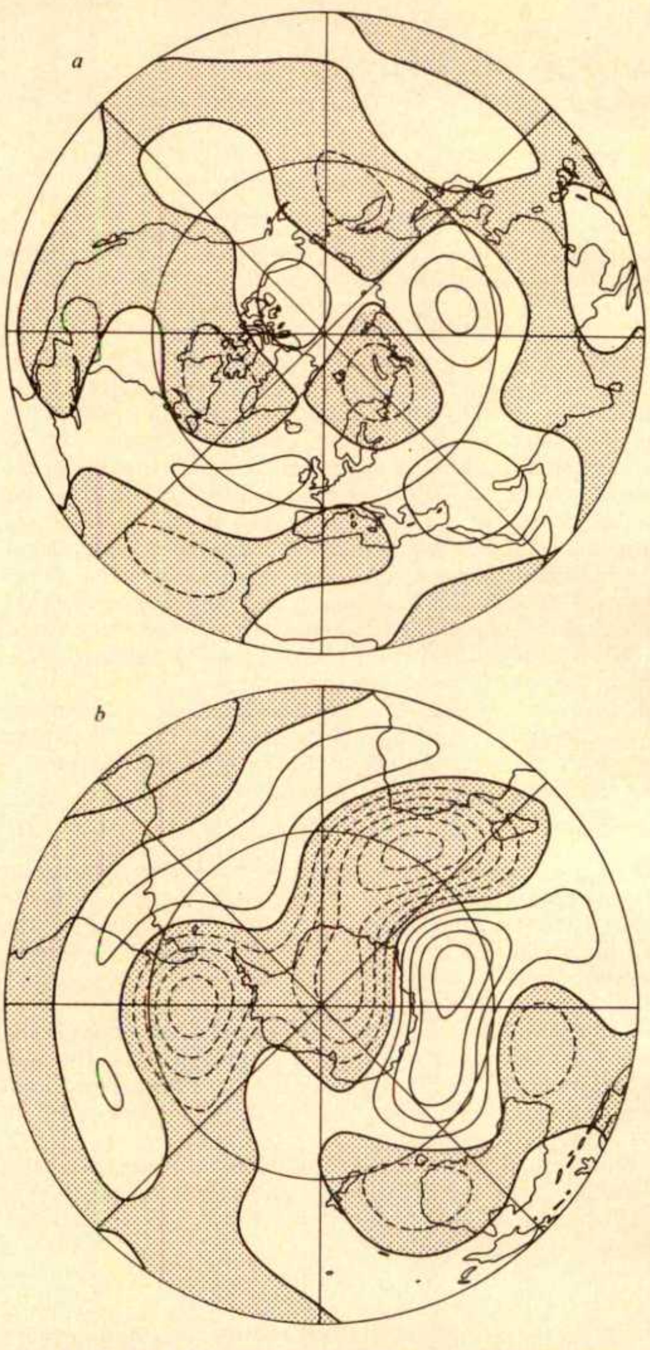

Fig. 3 As Fig. 2 but for the rate of change of field with time, epoch 1945, contour interval 1 μT per year. Negative values contribute to the fall in the Earth's dipole moment. Clearly most of the fall stems from the Southern Hemisphere (*b*); the Northern Hemisphere map (*a*) is relatively featureless. The fall is associated mainly with intensification and southerly movement of the reverse flux features (5, 6 in Fig. 2*b*).

two decades—in 1980 the two patches of reverse flux merged for the first time. Perhaps feature 6 will, in future, slow down as it crosses the south Atlantic.

The historical record is not long enough to allow study of a full cycle of these reverse flux features and their long-term development can only be speculated on. At no time is it possible to observe their reduction in size, merely their formation and intensification. Eventually they must vanish, possibly by combining with a flux patch of opposite sign during a phase of dipole growth such as occurred 4,000 years ago[4]. During the present phase of dipole decay it is reasonable to suppose that they will accumulate somewhere near the present position of feature 5, which in recent decades has slowed and gathered a more southerly motion.

Suppose these features form near Indonesia, drift west at the rapid rate of 0.3° yr^{-1}, intensify when they reach southern Africa, and then drift more slowly. Feature 5 would then have formed about 900 years ago, it was followed some 700 years later by feature 6, and perhaps there will be another in 500 years time. The time span of two or three such features is enough to cover the observed decay phase of the dipole moment[4].

Any further speculation as to the formation or demise of these features will need a theoretical study. Flux patch 6 and the one to the north-west resemble sunspots and may have a similar origin[5]. The sunspot cycle has been modelled successfully with dynamo waves[8] and a similar interpretation proposed for secular variation[9]. There are two distinct differences between these core spots and sunspots: the pairs form aligned north-west to south-east rather than laterally, which may indicate a weak toroidal field in the Earth's core[10], and they propagate polewards rather than towards the Equator. Both differences may eventually be explained by a theoretical study including the dynamo itself—indeed poleward propagation is preferred in some theoretical models[11].

The dynamo wave theory is attractive in providing a link with reversals. Kinematic $\alpha\omega$-dynamos exhibit either steady or periodic reversal behaviour, depending on details of the dynamo model[12]. The solar dynamo is well modelled by a periodic dynamo. The Earth may have a steady dynamo with superimposed waves driven by core-mantle boundary features such as topography beneath Indonesia or the hot region beneath southern Africa. At times of large amplitude the waves may induce a polarity reversal by a mechanism similar to that of a periodic $\alpha\omega$-dynamo. The reversal could only occur occasionally when fluctuations led to an abnormally large set of reverse-flux features, as envisaged by Cox[10].

Consider the consequences of this idea for geomagnetic polarity reversals. Each reversal results from secular variation features that are tied to the mantle. Local mantle temperature anomalies, like the one beneath southern Africa, change on the overturn time for mantle convection (~100 Myr). Therefore contemporaneous reversals should have similar characteristics (similar transition fields, for example). There is some palaeomagnetic evidence for this[13]. Any change in the mantle will lead to a change in secular variation characteristics, and consequently the nature and frequency of reversals.

At present the hot region at the base of the mantle lies in mid-latitudes at 40° S. A toroidal field in the core, generated by differential rotation acting on the predominantly dipolar field, will be strongest at mid-latitudes and zero at the poles and Equator[14,15]; the hot region is therefore ideally located for expulsion of flux and production of reverse-flux features. This accounts for the present large variations in dipole moment and possibly for the high occurrence of reversals in the last 40 Myr. If the hot region had been weaker in the past, or located over the poles or some other region unfavourable for the production of toroidal field, then secular variation would have been lower and reversals less frequent.

Changes in the frequency of geomagnetic reversals are well documented[16]. The rate at which reversals occur has increased almost linearly[17] during the past 86 Myr since the Cretaceous quiet interval when there were no reversals. It is suggested that this increase in reversal frequency is associated with the growth (or possibly the migration) of the hot region presently beneath southern Africa and the secular variation features it has driven.

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Analysis of multiexponential functions without a hypothesis as to the number of components

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The analysis of a curve as a sum of exponentials is one of the most common problems encountered in experimental 'curve-fitting' in such diverse areas as electrophysiology, chemical kinetics, electrical engineering and nuclear science. In this paper, a new method for the analysis of multicomponent exponential functions (either real and/or complex) is presented. This method is based upon the combined use of the Laplace transform and of Padé approximants. As compared with approximation procedures, it does not require an *a priori* hypothesis as to the number *n* of components, which is an output of the analysis[1]. It thus becomes possible, under realistic numerical conditions, to address the problem of unambiguous detection of the exponential components. Performance comparisons show that several practical limitations reported in previous works are overcome. Detailed derivations relevant to the fundamental basis of the method described here are presented in ref. 2 and further developments pertaining to numerical analysis aspects may be found in ref. 13.

We shall consider in this paper functions of the form:

$$f(t)=\sum_{j=1}^{n} A_j\, e^{\mu_j t} \tag{1}$$

where, in the most general case, the μ_j are complex numbers

$$\mu_j = \lambda_j + i\omega_j$$

with i denoting the imaginary unit,

When the μ_j are purely imaginary ($\lambda_j = 0$), the problem of the extraction of the different periodic components from the sum (1) is solved by the use of the classical Fourier transform which generates n peaks corresponding in position and amplitude to the n components present in $f(t)$. Thus this method allows the detection of the periodic components with no *a priori* hypotheses as to the number n. If on the other hand we consider real μ_j values (with possibly damped oscillations for $\omega_j \neq 0$) the problem of components detection (similar on the surface to the previous one) has proved in actual practice to be much more difficult due to the sensitivity of the exponents and amplitudes to small changes in the data (in mathematical terminology these functions display non-orthogonal behaviour as compared to the case of purely periodic components). However, by analogy with Fourier's approach, numerous methods have been elaborated relying on the generation of peaks in spectral functions for the detection of the exponential components with real exponents. The general scope of these methods amounted in fact to performing (directly or indirectly) the inversion of the Laplace transform of $f(t)$[3-6]. Severe limitations were however reported for these approaches, noticeably the presence of unresolved peaks for 'noisy' data. As a matter of fact, the practical difficulties encountered in the general problem of the evaluation of the inverse Laplace transform $\mathcal{L}^{-1}[f]$, for any $f(t)$, are now well-known[7]. Consequently, several workers have stated that the detection of exponential components inevitably leads to ill-posed problems and that methods for the analysis of functions such as (1) should address only the problem of function approximation. A function of unknown composition is approximated by k, $k+1$ ... exponentials and an 'optimal' value k_{opt} is determined, usually by statistical least-squares procedures. Now, Lanczos has provided examples in which a function sum of three exponentials could be reproduced to within two decimal places by a theoretical expression, a function sum of two exponentials, with significantly distorted time constants and amplitudes[8]. Following his pessimistic conclusions, it was generally accepted that an 'unrealistic accuracy' was required when using such procedures to recover the genuine exponential components.

The alternative method presented here allows the detection of exponential components with real and/or complex exponents under reasonable experimental conditions[1,2]. We resort to the Laplace transform $\mathscr{L}[f]$ of $f(t)$, which is indeed associated in a natural way with exponential functions, instead of trying to solve the problem of the inversion of this transform. Rather, we recover the exponential components present in $f(t)$ by evaluating the analytic expression of the Laplace transform for a function $f(t)$ which is known only through numerical tabulated values.

The Laplace transform is expressed in the form:

$$\mathscr{L}[f](p)=\int_0^{\infty} e^{-pt}f(t)\,dt \qquad (2)$$

where the variable p is a complex number. When applied to equation (1) this transformation leads to:

$$\mathscr{L}[f](p)=\sum_{j=1}^{n}\frac{A_j}{p-\mu_j} \qquad (3)$$

If for an experimental function $f(t)$ known at sampled intervals t_0, t_1, $t_2\ldots$ the above analytic expression (with respect to p) of $\mathscr{L}[f]$ was available, the problem of the exponential components detection could be solved by a mere identification of the poles of the rational expression (3). In order to achieve this goal we first evaluate by numerical integration the value at some point p_0 of $\mathscr{L}[f](p)$, together with the values of its successive derivatives

$$\frac{d^j\mathscr{L}[f]}{dp^j}(p)=\int_0^{\infty}(-t)^j f(t)\,e^{-pt}\,dt$$

up to some order K, thereby obtaining a local representation of the Laplace transform at the point p_0 by the corresponding Taylor series truncated to the order K

$$\mathscr{L}[f](p)\simeq\sum_{j=0}^{K}c_j(p-p_0)^j$$

with

$$c_j=1/j!(d^j\mathscr{L}[f]/dp^j)(p_0)$$

The crucial point of the method is to derive the construction of the desired analytical representation through the use of Padé approximants[9-11]. Padé approximants are well suited for this purpose, because according to equation (3) the Laplace transform is a rational function; in principle, therefore, it may be representedd exactly in the whole complex plane by a Padé approximant. Hence we use the name 'Padé-Laplace' to designate this new method of analysis.

A Padé approximant of the variable p is a rational function denoted $[L/M](p)$ obtained by the division of two polynomials $A_L(p)$ and $B_M(p)$ of degree L and M respectively:

$$[L/M](p)=\frac{A_L(p)}{B_M(p)}=\frac{\sum_{s=0}^{L}a_s p^s}{\sum_{v=0}^{M}b_v p^v}.$$

The basic feature of the Padé approximants which makes them appropriate for deriving an analytic expression for a function $F(p)$ knowing only a limited number of terms of its series expansion is that the power series expansion of the rational function $[L/M](p)$ is required to agree with that of $F(p)$ up to the term p^{M+L}. Accordingly, to achieve the construction of $[L/M](p)$, one needs the first $L+M+1$ coefficients of the Taylor series of $F(p)$. In a strictly theoretical frame, if the

Table 1 Analysis of a theoretical function $f(t)$

	Re μ_k	Im μ_k	Re A_k	Im A_k
Poles and amplitudes of Padé approximant [0/1]				
1	−0.00691	0.00000	185.15	0.00
Poles and amplitudes of Padé approximant [1/2]				
1	−0.00404	0.00000	150.93	0.00
2	−0.05271	0.00000	48.35	0.00
Poles and amplitudes of Padé approximant [2/3]				
1	−0.00400	0.00000	150.09	0.00
2	−0.04662	0.00000	40.09	0.00
3	−0.08651	0.00000	9.51	0.00
Poles and amplitudes of Padé approximant [3/4]				
$a\to 1$	−0.00400	0.00000	150.00	0.00
$b\to 2$	−0.04000	0.00000	19.01	0.00
$c\to 3$	−0.06001	0.00000	29.99	0.00
$d\to 4$	−0.40172	0.00000	1.00	0.00
Poles and amplitudes of Padé approximant [4/5]				
$a\to 1$	−0.00400	0.00000	150.00	0.00
$c\to 2$	−0.06001	0.00000	29.98	0.00
$b\to 3$	−0.04001	0.00000	19.02	0.00
4	0.09178	0.00000	0.00	0.00
$d\to 5$	−0.40240	0.00000	1.00	0.00
Poles and amplitudes of Padé approximant [5/6]				
$a\to 1$	−0.00400	0.00000	150.00	0.00
$b\to 2$	−0.04000	0.00000	18.99	0.00
$c\to 3$	−0.05999	0.00000	30.01	0.00
4	0.00966	−0.04444	0.00	0.00
5	0.00966	0.04444	0.00	0.00
$d\to 6$	−0.40038	0.00000	1.00	0.00

The analytic expression of the function analysed was: $f(t)=f_a(t)+f_b(t)+f_c(t)+f_d(t)=150\exp(-0.004t)+19\exp(-0.04t)+30\exp(-0.06t)+\exp(-0.4\,t)$ for $t=0,1,2,\ldots,1199$. In order to be close to theoretical conditions (no external noise added and minimization of the computational noise) 1,200 points were generated on the computer and the values were rounded at the fifth decimal. The panel displays the output of the analysis in the form of the poles and the amplitudes of the Padé approximants $[(L-1)/L]$ of increasing order. For each approximant $[(L-1)/L]$ the parameters of the component k (with exponent μ_k and amplitude A_k) appear at the line whose index is k in the following order: Re μ_k, *Im* μ_k, *Re* A_k, *Im* A_k. As discussed earlier the effective parameters are obtained from the approximant [3/4]. The first artificial root appears in [4/5] at the rank $k=4$ with an amplitude of about 2.2×10^{-6} (this amplitude becomes zero with the number of digits chosen here for the table and is in a ratio of 45.5×10^4 with the smallest amplitude $A_d=1$ and in a ratio of 68.2×10^6 with the amplitude $A_a=150$). As a difference from the genuine roots this 'component' with a positive exponent is not stable and disappears in [5/6], splitting into a pair of complex conjugate roots with negligible amplitudes. Spurious roots which give in theory zero amplitudes correspond to an important theorem due to Padé and further developed by Chisholm and Baker[15,16]. According to this theorem when the initial function is a rational function $F(p)=P_n(p)/Q_m(p)$ with n and m the degrees of the numerator and the denominator respectively, then the Padé approximant $[n/m]$ gives back exactly the initial function $F(p)$ and all higher order approximants $[N/M]$ (with $N\geqslant n$ and $M\geqslant m$) reduce to this $[n/m]$ exact expression. The example displayed allows comparisons with previous methods: (1) in the methods relying on the generation of peaks in spectral functions, the height of the peaks are governed by the values of the parameters A_k/μ_k which need to be in the same order of magnitude in order to get a satisfactory resolution[4]. We have chosen the inverse constraint as $A_a/\mu_a=37{,}500$ and $A_d/\mu_d=2.5$. The component d is very poorly taken into account in the data as its amplitude represents only 0.5% of the total while due to its very small time constant (100 times smaller than that of the component a) it decreases to 0 within very few time steps. These parameters originate from the analysis of correlation functions from patch-clamp data with the presence of components associated with very fast kinetics phenomena[1,13] (manuscript in preparation). (2) The components b and c are such that $\mu_c/\mu_b=1.5$. This ratio is much closer to the theoretical limit value 1 for the resolution of two exponentials than the value 2.5 reported by several workers, as a practical limit[6]. The calculation of the coefficients of the Padé approximants for a given set of c_j values was performed according to an algorithm of Longman[17]. For the calculation of the integrals c_j (see text) several methods were used and compared: a 48-point Gauss–Laguerre formula[18], the trapezoidal rule and Simpson's integration rule which improved the efficiency of the method for analyses involving a large number of components or a high noise level (see Fig. 1). Recently we devised a variant of Simpson's rule after an idea of Filon[19,20] and performed an analytic integration of the terms $(-t)^k\exp(-p_0t)$ in the integrals c_j thus restricting the parabolic interpolation to the function $f(t)$ for which the Laplace transform is calculated. Another important point with integration methods such as Simpson's is the possibility of choosing a new time unit, thus getting a new value (denoted by ΔT) for the integration step. It actually appears that this choice is critical, the optimal value depending on the number N of points (ΔT was set to $1/\sqrt{N}$) (ref. 13), the technical details of which will be presented elsewhere.

Table 2 A famous example from Lanczos revisited

	Re μ_k	Im μ_k	Re A_k	Im A_k
	Poles and amplitudes of the Padé approximant [0/1]			
1	−3.8617	0.0000	9.9407	0.0000
	Poles and amplitudes of the Padé approximant [1/2]			
1	−1.7241	0.0000	1.5489	0.0000
2	−4.5867	0.0000	8.4504	0.0000
	Poles and amplitudes of the Padé approximant [2/3]			
$a\to 1$	−0.9962	0.0000	0.4015	0.0000
$b\to 2$	−3.0162	0.0000	3.4435	0.0000
$c\to 3$	−5.0086	0.0000	6.1550	0.0000
	Poles and amplitudes of the Padé approximant [3/4]			
1	1.3183	0.0000	0.0000	0.0000
$a\to 2$	−0.9593	0.0000	0.3711	0.0000
$b\to 3$	−2.9593	0.0000	3.3361	0.0000
$c\to 4$	−4.9872	0.0000	6.2929	0.0000
	Poles and amplitudes of the Padé approximant [4/5]			
1	6.5216	0.0000	0.0000	0.0000
2	2.3722	0.0000	0.0000	0.0000
$a\to 3$	−0.9826	0.0000	0.3893	0.0000
$b\to 4$	−2.9914	0.0000	3.3925	0.0000
$c\to 5$	−4.9986	0.0000	6.2182	0.0000

We consider the function $f(t)=A_a\exp(-t)+A_b\exp(-3t)+A_c\exp(-5t)$ originally presented by Lanczos[8]. We normalize parameters by setting $A_a+A_b+A_c=10$, multiplying each A_i by a factor of about 4: $A_a=0.4$, $A_b=3.4$ and $A_c=6.2$. We mimicked Lanczos' conditions by generating 24 sampled $f(t)$ values rounded to the second decimal, with the only difference being that the sampling step is set to 0.15 instead of 0.05 in order to ensure a full decay to 0 for $f(t)$. The output of the computer analysis shows with no ambiguity that in our framework the procedure of detection is effective. The approximation with two exponentials provided by the approximant [1/2] allows a reconstitution of the initial data with a standard root mean square deviation of 0.01. It was in fact the possibility of such a fit to the data with two exponential components, with distorted parameters, which was the basis of Lanczos' pessimistic conclusion. With such sparse data (the time constants are of the order of magnitude of the sampling step) the numerical integration in the Laplace transformation would introduce too large an error due to the parabolic interpolation. The analysis shown was thus performed with one of the variants allowed by the generalization of the framework provided by our method. We define a functional transformation well-adapted for the identification of some type of components f_i. In the logic of our approach such a transformation $\mathcal{T}[f]$ (with $f=\sum_i f_i$) should allow the detection of the components f_i as poles of the analytic expression of $\mathcal{T}[f]$, derived from its Taylor series expansion by use of Padé approximants (appendix by P.C. in ref. 1) (ref. 2). Here, a well-adapted transformation is the z-transform which may be viewed as a discrete version of the Laplace transform. The well-known Prony method for the analysis of multiexponential functions can be reformulated as a [Padé]-[z-transform] method with the constraint that the parameter z_0 for which the Taylor series expansion is calculated is placed at infinity[21]. For the above example, with this choice for z_0, the Padé approximants of successive orders yielded a representation with two exponentials whose parameters are in agreement with those of the approximation with 2 exponentials provided by Lanczos. In marked contrast, with a suitable finite value for z_0 (here $z_0=2$), the three genuine components are recovered in the analysis displayed in the table at orders higher than [1/2] (the approximation with two exponentials obtained at [1/2] is again essentially equivalent to the one proposed by Lanczos). This feature clearly illustrates the increase in detection power gained through the allowance for an optimal choice for the value of z_0 (or, correspondingly, for the parameter p_0 in the Padé–Laplace procedure).

Table 3 Padé–Laplace analysis of the curves in Fig. 1

	Re μ_k	Im μ_k	Re A_k	Im A_k
	Poles and amplitudes of Padé approximant [3/4]			
1	−0.00222	0.00000	464.24	0.00
2	−0.00837	0.00000	−261.70	0.00
3	−0.00553	−0.02580	97.43	−71.65
4	−0.00553	0.02580	97.43	71.65
	Poles and amplitudes of Padé approximant [4/5]			
$a\to 1$	−0.00200	0.00000	400.00	0.00
$c\to 2$	−0.02000	0.00000	−500.00	0.00
$b\to \{3$	−0.00800	−0.03000	175.00	0.00
4	−0.00800	0.03000	175.00	0.00
$d\to 5$	−0.20002	0.00000	250.02	0.00
	Poles and amplitudes of Padé approximant [5/6]			
$a\to 1$	−0.00200	0.00000	400.00	0.00
$c\to 2$	−0.02000	0.00000	−500.01	0.00
3	−0.04614	0.00000	0.03	0.00
$b\to \{4$	−0.00800	−0.03000	175.00	0.00
5	−0.00800	0.03000	175.00	0.00
$d\to 6$	−0.20004	0.00000	250.01	0.00
	Poles and amplitudes of Padé approximant [8/9]			
1	0.00995	0.00000	0.00	0.00
$a\to 2$	−0.00200	0.00000	400.00	0.00
$c\to 3$	−0.02000	0.00000	−500.00	0.00
$b\to \{4$	−0.00800	−0.03000	175.00	−0.01
5	−0.00800	0.03000	175.00	0.01
6	0.00019	−0.01785	0.00	0.00
7	0.00019	0.01785	0.00	0.00
$d\to 8$	−0.19998	0.00000	249.86	0.00
9	−0.61936	0.00000	0.55	0.00
	Poles and amplitudes of Padé approximant [10/11]			
$a\to 1$	−0.00200	0.00000	398.92	0.00
2	0.00209	−0.01182	−0.03	0.00
3	0.00209	0.01182	−0.03	0.00
$c\to 4$	−0.02074	0.00000	−512.85	0.00
$b\to \{5$	−0.00800	−0.02995	177.79	−0.45
6	−0.00800	0.02995	177.79	0.45
7	0.01191	−0.03198	0.00	0.00
8	0.01191	0.03198	0.00	0.00
9	0.00322	−0.05576	−0.04	−0.22
10	0.00322	0.05576	−0.04	0.22
$d\to 11$	−0.19600	0.00000	264.66	0.00
	Poles and amplitudes of Padé approximant [11/12]			
1	0.00122	0.00000	−0.09	0.00
$a\to 2$	−0.00201	0.00000	401.67	0.00
3	0.00348	−0.02315	0.02	0.00
4	0.00348	0.02315	0.02	0.00
$c\to 5$	−0.02009	0.00000	−506.57	0.00
6	0.00039	−0.00929	0.06	0.12
7	0.00039	0.00929	0.06	−0.12
$b\to \{8$	−0.00791	−0.03004	174.41	1.58
9	−0.00791	0.03004	174.41	−1.58
10	0.01047	−0.06043	0.04	−0.05
11	0.01047	0.06043	0.04	0.05
$d\to 12$	−0.19738	0.00000	262.22	0.00

original function $F(p)$ is a rational function of degree $N-1$ for the numerator and N for the denominator (as in the function $\mathcal{L}[f](p)$ in equation (3) after reduction to the same denominator), then the corresponding functions $[L/M](p)$ will be 'approximants' only up to order $[(N-2)/(N-1)]$, because the Padé approximant $[(N-1)/N]$ will in fact give the exact analytic expression of $F(p)$.

In order to illustrate the way in which the components detection is achieved through this procedure, let us first consider a theoretical function $f(t)$, sum of four exponentials (Table 1, which also gives complementary information relevant to aspects of our numerical analysis). We consider the successive Padé approximants $[(L-1)/L]$ resulting from a number of Taylor coefficients of the Laplace transform of $f(t)$. First, the Padé approximinant [0/1] provides (through its pole and the corresponding residue) an approximation of the original function with a single exponential; second, the Padé approximant [1/2] provides (through its two poles obtained as roots of the denominator and the corresponding residues) an approximation of the original function with two exponentials; the Padé approximant [3/4] gives the exact theoretical expression of the original function. It is evident that in this analysis the series of roots in the successive denominators will not display any common component. Because, in general, one will not know the expression of the original function, and not even the number of components of this expression, one must pursue this analysis further. However, for the specific example considered it will be sufficient to obtain the [4/5] approximant, because it must reduce to the [3/4] approximant, and this will remain true for all approximants of higher orders. That is, the [4/5] approximant will display with no modifications the four components already detected in the [3/4] approximant, and also an additional component—which may be called an 'artificial component' (see caption of Table 1)—corresponding to the multiplication of the numerator and the denominator by an equal factor. This component will have a zero amplitude A_j in the partial fraction expansion (3). In conclusion, if one is interested only in obtaining an approximation of the function analysed with L components, one may restrict the analysis to the $[(L-1)/L]$ approximant. On the other hand, if the problem of detection is

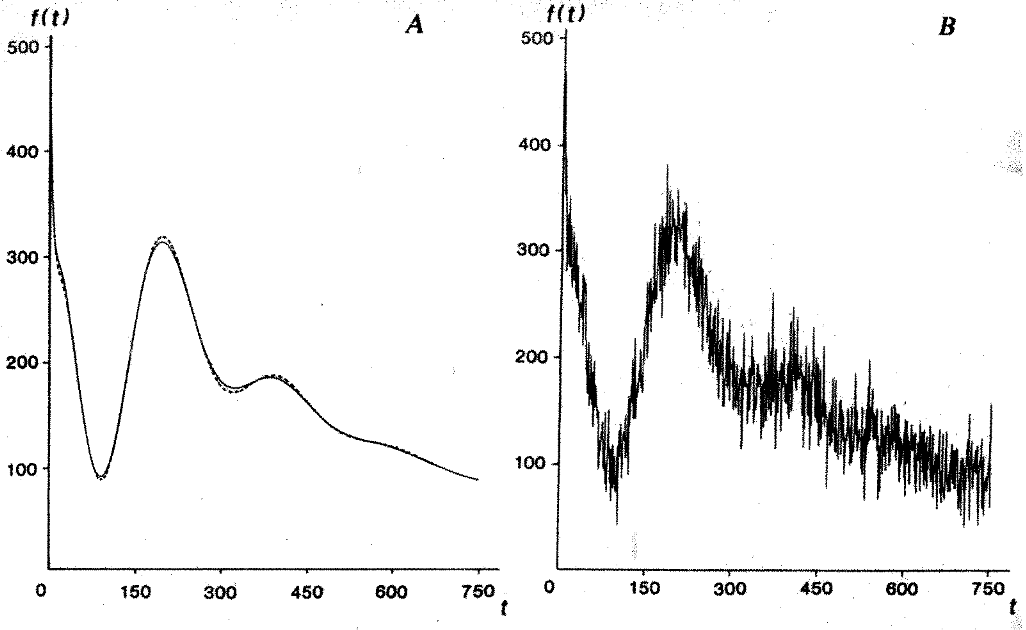


Fig. 1 Application to simulated data with gaussian noise (sum of exponentials with real exponents and one cosine component). The analytical function $f(t)$ generated on the computer was $f(t)=f_a(t)+f_b(t)+f_c(t)+f_d(t)=400 \exp(-0.002t)+350 \exp(-0.008t) \cos(0.03t)-500 \exp(-0.02t)+250 \exp(-0.2t)$ and is represented in panel *A* (solid line). Gaussian noise with mean zero and a standard deviation value of 25 was added to $f(t)$, the resulting 'noisy function' is represented in *B*. The level of noise (ratio of the standard deviation value to the amplitude of each component) ranged between 5% (component *c*) and 10% (component *d*). The components detection was first performed on the data without noise (3,000 sampled points for $t=0, 1, 2, \ldots$; value chosen for the integration step $\Delta T=0.01826$; $p_0=0.89$) and the resulting output is displayed in Table 3 (approximants [3/4] to [8/9]). The 4 components labelled a, b, c and d are detected in the approximants [4/5] and [5/6]. The first artificial root which appears in [5/6] has an amplitude of the order 3×10^{-2}. It should be noted that using the complex exponential notation, the component $f_b(t)$ is displayed as the sum of two conjugate roots $f_b(t)=175 \exp(-0.008t)[\exp(i0.03t)+\exp(-i0.03t)]=350 \exp(-0.008t) \cos(0.03t)$. The resulting curve is drawn in *a* and is indistinguishable from the theoretical curve $f(t)$. In order to demonstrate the stability of the analysis, an approximant of high order ([8/9]) is also displayed. The quality of the analysis was slightly affected when a severe truncation was performed, taking into account only the 750 points represented in *a* (for example, the amplitude for $f_c(t)$ was -499.45 against -500). The output of the analysis performed on the data with noise in the same conditions as for the curve without noise is also shown in Table 3 (approximants [10/11] and [11/12]). The four components are again detected, the slight numerical alteration affecting mainly the component d having the smallest amplitude. The curve corresponding to the analytical expression derived from approximant [11/12] (Table 3) is drawn in *A* (dashed). The analysis and detection were also efficient for gaussian noise having a standard deviation of 50 with, however, a rather significant alteration for the amplitude of the component $f_d(t)$ (334 against 250).

to be solved with no *a priori* hypotheses as to *n*, a step-wise procedure would be adopted, in which the Padé approximants of increasing orders are considered, the detection being achieved when this procedure is pursued up to order $[L/(L+1)]$ (at which one 'artificial root' appears for the first time). At that point, the previous approximant $[(L-1)/L]$ is equal to the exact Laplace transform $[(n-1)/n]$, and it therefore gives the desired exponents and amplitudes. The numerical realization of this theoretical scheme is illustrated by the computer output given in Table 1. On the grounds of performance comparisons for theoretical functions, the example analysed shows also that severe limitations reported in several previous works for the separation and detection of exponential components are overcome by the procedure. Theoretical comparisons with other methods can be found in ref. 2.

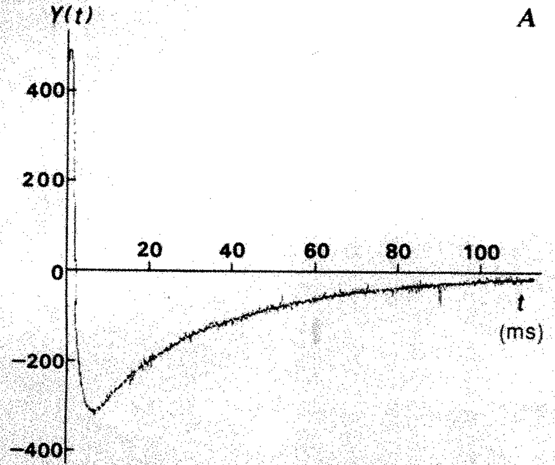
Next, we further assess the practical effectiveness of the detection procedure in more realistic cases. First, it was worth revisiting in Table 2 the famous example of Lanczos, already mentioned above[8], which led the author to the extremely pessimistic conclusion (reiterated ever since by researchers in the field) that the components separation problem is so 'skew-angular' and encounters 'such unsurmountable difficulties' that the only remedy would be an 'increase of accuracy' to 'completely unrealistic' limits[8]. It would not be advisable in a genuine experimental case to address the identification problem with such poor information (as for the function in Table 2 derived from Lanczos' treatment) nevertheless, we believe that this example allows a significant shift in the limits which had to be set for the validity of such analyses.

This example, through a comparison with a well-known method for the analysis of multiexponential functions, namely the one proposed by Prony in 1795[12], also highlights an essential practical feature of our approach. In a strictly theoretical framework, the detection procedure described is effective for any p_0 value. However, numerical investigations indicated that in practice the identification of components could be performed only for p_0 values lying in some 'optimal' interval, denoted by I_f (refs 2 and 13). In fact, it is not surprising that the value chosen for p_0 (which is the only input parameter of the method) can significantly affect the numerical precision in the calculation of the integrals c_j defining the coefficients of the Taylor series expansion of $\mathscr{L}[f]$. We examined the theoretical and numerical aspects of this problem, in order to implement an automatic procedure of analysis[13]. It appears that the optimal choice for p_0 is one such that the coefficients c_j are of the same order of magnitude. The general idea is thus to set (by varying the free parameter p_0) the centre of the convergence disk of the Taylor series of $\mathscr{L}[f]$ at a distance equal to 1 from the pole μ_1 of the Laplace transform which lies closest to 0. It is clear that on the other hand extreme p_0 values will lead to loss of computational accuracy. The detailed criterion and the iterative automatic procedure elaborated for the choice of p_0 will be discussed elsewhere.

Table 3 illustrates the effect of the addition of artificial gaussian noise to an analytic function. The data show that, despite high noise levels (see Fig. 1 for a graphic representation of this level), the components detection and analysis still perform satisfactorily (Table 3). The example treated also demonstrates the ability of the method to detect accurately the complex components.

In Fig. 2, two experimental applications of the method are displayed. In the example shown in Fig. 2*d*, the method (as a method of approximation) was deliberately applied to a curve which is not a sum of exponentials, namely $Y(J)=J^{-3/2}$, in order to obtain an analytic expression of this curve with the desired number of exponential components. Other applications include the analysis of chemical relaxation signals[14] and electrophysiological signals from cardiac cells (manuscript in preparation, see Fig. 2).

In conclusion, in each case of practical applicaton, numerical simulations such as those discussed above could allow an assessment of the limits of validity of the detection procedure for exponential components (the limit of resolution of the exponents for a given accuracy or noise level). Also from a practical point of view, it becomes possible to implement on-line procedures which do not need the technical skill demanded by many sophisticated nonlinear least-squares procedures. From the theoretical point of view a rational generalization of the framework



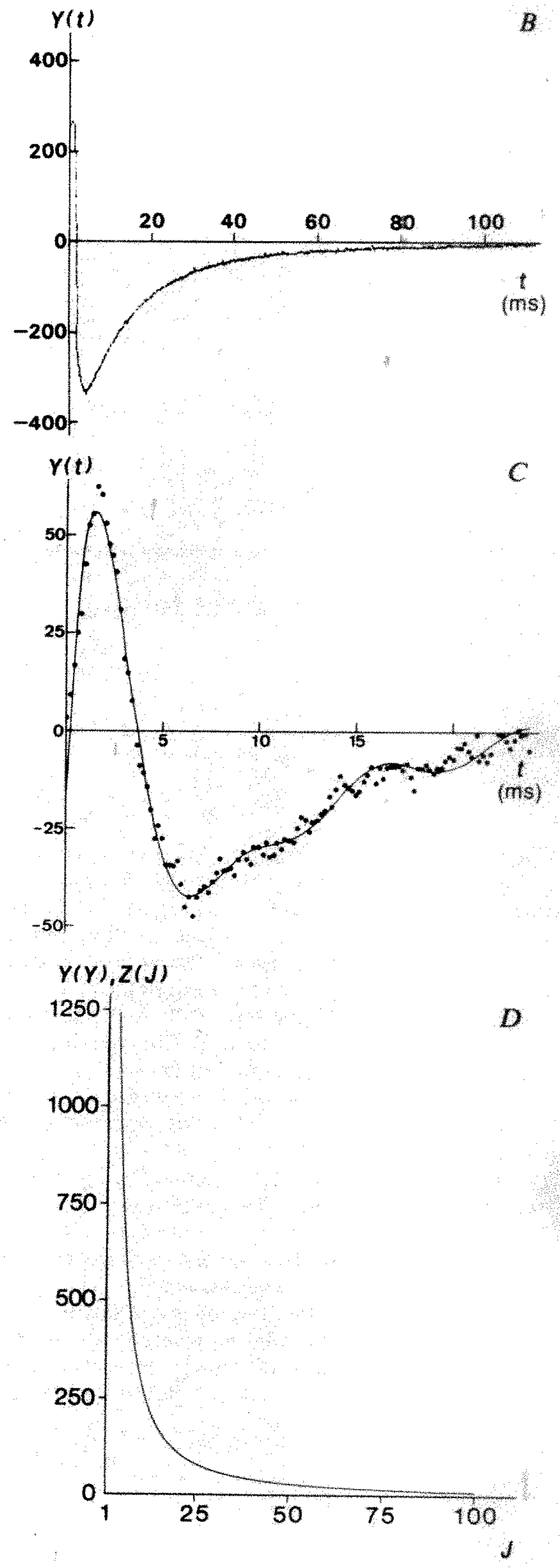


Fig. 2 Examples of application to experimental data and to approximation problems. *A*, *B*, In this application, performed at the Unité INSERM U-241 (Laboratoire de Physiologie Cellulaire Cardiaque, Orsay, with R. Fischmeister and P. Lechene) on electrophysiological signals from cardiac cells, the object was to determine the effects of drugs and membrane potentials on the different components of the observed membrane currents. The two experimental curves in *A* and *B* exhibit different decay behaviours. The components detection using the Padé–Laplace method showed that in both cases there were three exponentials with the same time constants ($\tau_a \approx 44.42$ ms, $\tau_b \approx 12.16$ ms and $\tau_c \approx 1.14$ ms in *A*; and $\tau_a = 44.6$ ms, $\tau_b \approx 14.3$ ms and $\tau_c \approx 1.9$ ms in *B*). The apparent difference in this example was because of a consistent modification in the magnitudes of the amplitudes. In order to verify the quality of the 'fittings', the analytic expressions obtained were plotted by computer (continuous lines), together with the experimental data. *C*, This example is a recording obtained at the Unité INSERM U-261 (Laboratoire de Neurobiologie Cellulaire, Institut Pasteur). The object was to investigate the different components involved in the synaptic potentials (e.p.s.p. – i.p.s.p. sequence) of a relay neuron from a thalamic preparation *in vitro*. The example illustrates the ability of the method to yield satisfactory analytic expressions of complex curves. However, the example also indicates the limitations of the detection method: that is, owing probably to the noise level and the small (as compared with the number of components in the curve) number of data points, the analytic expression obtained for the complete curve was not unique (no I_f interval displayed by the analysis and no appearance of stable roots). The specific analytic expression drawn as a continuous line was selected based on a 'goodness of fit' criterion. *D*, In the theoretical treatments of DNA thermal denaturation experiments one must—in order to consider the internal denucleation phenomenon—attribute statistical weights for the loops formed when k base pairs are broken. Usually, the weight functions used are of the form $w(k) = (2k+2)^{-\beta} = j^{-\beta}$ (β being often set to 3/2). It is well known[22] that the computer times for the calculation of the theoretical denaturation curves can be drastically reduced (proportional to n instead of n^2, n being the total number of base pairs in the DNA molecule) if one approximates the $w(k)$ function by a sum of exponentials. Such an application was performed in collaboration with F. Schaeffer and B. Caudron at the Institut Pasteur. The curve $Y(J)$ shown for J between 4 and 100 was $Y(J) = 10^4 \times (J)^{-3/2}$. The approximation $Z(J)$ with 10 exponentials (for J up to 2,000) obtained for this curve with the Padé–Laplace method was also drawn, but with the scale of the figure it could not be distinguished from the curve $Y(J)$. A more accurate comparison between $Y(J)$ and $Z(J)$ for some J values can be gained from:

J	$Y(J)$	$Z(J)$	J	$Y(J)$	$Z(J)$
10	316.228	317.832	201	3.50918	3.50835
11	274.101	275.527	202	3.48315	3.48233
12	240.562	241.579	450	1.04756	1.0473
50	28.2842	28.2758	451	1.04408	1.04382
51	27.4564	27.4485	452	1.04062	1.04035
52	26.6682	26.6608	1.000	0.316228	0.316143
200	3.53553	3.5347	1,001	0.315754	0.315669
			1,002	0.315281	0.315196

elaborated[2] could allow one to address various problems in the field of models identification, such as the detection of lorentzian or gaussian components and possibly global analyses of certain two-dimensional data (by use of two-dimensional Padé approximants). Work in these fields is in progress.

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New explanation for chain folding in polymers

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Polymer crystals are characteristically thin in the molecular direction[1] so that materials such as polyethylene and nylon have an extremely finely divided texture consisting of crystal lamellae interspaced with non-crystalline regions (melting points are depressed by 20 °C or more). Many distinctive properties of these polymers follow as a consequence of this phenomenon, often known as chain folding because molecules generally fold back and forth across the thin dimension of the crystals. The currently accepted explanation of the origin of chain folding in terms of 'nucleation' ('LH' theories[2]) appears to be inconsistent with the observed shapes of crystals[3] (see also Wunderlich[4]). In this paper I describe how an approximate treatment of an alternative model[5,6] involving rough growth surfaces and molecular 'pinning' can give analytical results in line with experiment. The aim is to derive the result in such a way as to concentrate on the physical mechanisms involved and to discuss the kinetic barriers caused by molecular continuity. If there turns out to be any connection between chain folding of synthetic molecules and folding in proteins, it will be clear that the models discussed here may be more readily extended to the latter than LH models[2], because LH models[2] are specific to crystals and not applicable, for example, to the ribbon structures which are formed from β sheets[7,8].

Polymer crystals are generally platelets (Fig. 1*a* and *b*). The thickness l is typically 4–120 lattice repeats ($\sim$5–50 nm) and only slightly greater than l_m, the thickness that would be in equilibrium with the liquid at the crystallization temperature

$$l_m = \varepsilon T_m^0 / h \Delta T \qquad (1)$$

$\varepsilon/2$ is the (fold) surface free energy per chain emerging at the fold surfaces, h is the latent heat of fusion per unit, T_m^0 the melting point of a crystal of infinite thickness, and ΔT the supercooling; l is measured in numbers of units each of several chemical repeats[3,9]. The concept of a unit allows in a simple, if non-rigorous way, for the connectedness of the monomers, such that they cannot move independently, and also for any stiffness of the chain. It follows from equation (1), and from l generally being close in value to l_m, that $l \sim \Delta T^{-1}$, the commonly observed trend[10,11].

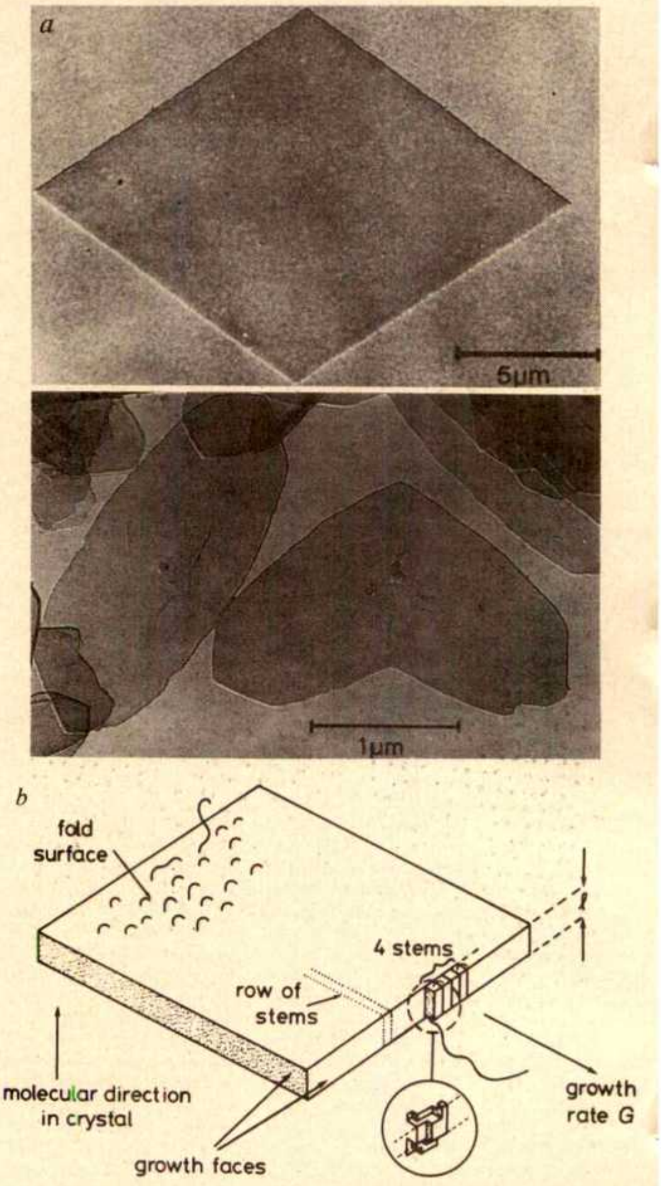
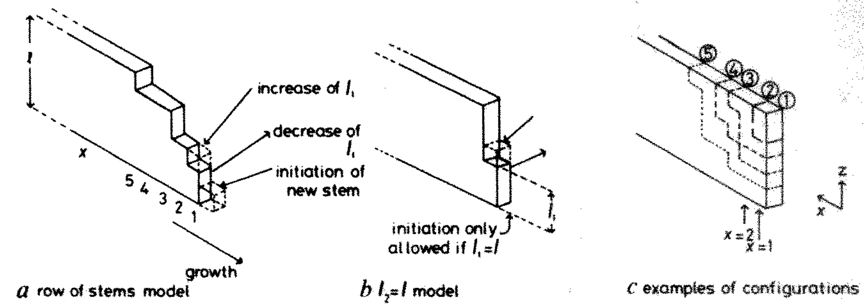


Fig. 1 *a*, Electron micrographs of polyethylene crystals, showing straight and curved shapes of outlines. In the latter case the crystals must have re-entrant corner sites on the perimeter (for example, at twin boundary of the twinned crystal), (courtesy of Dr S. Organ). Curved rather than straight outlines tend to occur for higher crystallization temperatures, which suggests a molecular roughness on the perimeter which increases with temperature[3]. *b*, Schematic representation of a polymer crystal indicating the chain direction, fold and growth surfaces, and the nature of a growth face step as envisaged by nucleation (LH) theories. The inset shows an alternative view of the region of the growth face involving a step[3,5]. In which case the stems at the growth face can be short. The details of the folding back (adjacent re-entry or otherwise[28]) are not of primary interest here: several types of folds and loops are included. The dotted lines refer to the structures in Fig. 2.

A kinetic theory for chain folding is one that predicts lamellae as the morphology corresponding to fast growth, so that the final state is generally metastable and not in equilibrium. We now consider why the speed of a growth front (growth rate G) for a given l value can then be written according to the following equation, for both nucleation and roughness-pinning models[12,13]

$$G \propto e^{-Kl}(l - l_m)\Delta f / kT \qquad (2)$$

Δf is the (bulk) change in free energy on crystallization ($h\Delta T/T_m^0$). The following elucidates how equation (2) is equivalent to those usually given for LH theories[2,9,14-22,29] where



a row of stems model

b $l_2=l$ model

c examples of configurations

Fig. 2 Representations of the two-dimensional models discussed in this paper (see dotted lines in Fig. 1*b*). *a*, The row of stems model, with molecular direction (z) vertical as in Fig. 1*b*. Vertical 'bond' energies ε can be associated with loops and partially attached chains (for example). Modification to the lengths of stems are only permitted at the outermost end of the row (that is, $x=1$, to the right). The length l_1 of the outermost stem may be increased or decreased by one unit. Stems at $x=1$ of length unity can be removed, and initiation of a new stem of one unit can be made adjacent to the previous outermost stem. The length l_2 of the next but one outermost stem can only be changed if the stem at $x=1$ is removed. *b*, In the $l_2=l$ model, only those configurations of the row of stems model are allowed where l_2 (the length at $x=2$) is equal to the final length l. These configurations are 'viable' for growth, whereas those in *a* with $l_2<l$ are not, because they must remove at least one stem before the configuration can evolve into one with all the stems in this part of the row being of length l. *c*, Examples of stem configurations, labelled 1-5. Configuration 1 (probability $PC(l)$) is the squared-off (non-tapered) one through which the system must pass before the stem in the position currently labelled $x=1$ can be incorporated in the body of the row crystal. Configurations 1 and 2 are described within the $l_2=l$ model (total probability P for all such). Configurations 3-5 are such as to block the movement of the growth point. The energies increase with the number of short stems. For example, 5, (dotted line) would have a relatively high energy and low probability. The total number of configurations, each one weighted according to its Boltzmann probability, can be calcuated using a random walk analogy (derivation of equation (4)). The principal assumptions and approximations of the row of stems model used here are given in the text and their limitations and justifications are discussed in more detail elsewhere. A list of these is as follows: neglect of correlations along the growth face between different rows; contributions to energy are accounted for by nearest neighbour 'bonds' in a two-dimensional lattice; the molecular continuity is accounted for by pinning rules (changes only at $x=1$); l is a uniform in fully grown row, $l_{x+1}\geq l_x$; the distribution of l_x near the growing end corresponds to equilibrium. The last approximation is made for mathematical simplicity only (see note in proof).

the 'barrier term' e^{-Kl} arises as the probability of overcoming the (largely enthalpic) free energy barrier involved with the creation of steps on the growth face. A step (Fig. 1*b*) has an associated free energy σ_n which, in LH theories, contains the product σal where σ is a side-surface free energy per area and al is the area exposed in the step. The crucial assumptions are: (1) σ_n increases linearly with l and (2) σ_n is generally large compared with kT. Growth rates are then calculated[18] as the product of the steady-state density of steps and the average rate at which steps spread. The exponent Kl is either $2\sigma_n/kT$ or σ_n/kT, depending on the substrate width (regimes I or II[19,18]). The term in equation (2) proportional to $(l-l_m)$ can be derived from the probability that patches can be removed, and from the step spreading rate. Such a term is clearly necessary to ensure $G=0$ at the melting point ($l=l_m$). Equation (2) gives a maximum in G with respect to l[15] (also see below in connection with Fig. 3), and as long as K is sufficiently large this maximum corresponds to l not much greater than l_m. The term e^{-Kl} for $l=l_m$, with l_m substituted from equation (1), then becomes $e^{-K_g/T\Delta T}$ (K_g being a constant) which has the same dependence on ΔT as is found for G experimentally[14] (the dependence of G on ΔT is dominated by the barrier term e^{-Kl}). There have been recent developments in LH theories[2] (for example refs 20-22), but they all retain the two basic assumptions concerning σ_n. For K to be sufficiently large for l and G values to be derived in this way, large σ_n values are essential (for example, 25 kT for polyethylene lamellae of thickness 30 nm). For any such high σ_n, re-entrant corner sites on the crystal perimeter should be very much favoured for addition of new material, (for example, by factors of $e^{2\sigma_n/kT}\approx e^{50}$ or more in polyethylene) because attachments elsewhere involve the creation of two steps with a consequent free energy barrier of $2\sigma_n$. Hence all crystal shapes should evolve very quickly during growth to give straight-sided convex polygons. In contrast, crystals with curved perimeters[3,23] and double crystals and twins with re-entrant corner sites[24] are commonly observed morphologies that are stable during growth (see also Fig. 1*a*). The assumption $\sigma_n\propto l$ in LH theories[2] is also at variance with estimates of σ_n from crystal shapes. In several cases where information is available, thicker lamellae tend to show more curvature than thin ones (for example, the lower part of Fig. 1*a* compared with the upper part). Hence, as curvature implies lower σ_n, morphology then suggests that σ_n tends to decrease (not increase) with l.

It has been shown that curved crystal outlines can be the consequence of rough growth faces[25]. If indeed there can be such a roughness for polymer crystals[3] there must be a barrier to the growth which is completely different from nucleation. The following represents a very simple form of the model, and shows how a barrier term of the form e^{-Kl} can arise. This is because in the model there is an entropic barrier to growth. The growth face explores many configurations, only a minority of which allow the face to progress. Most are in effect blocked by the kinetic constraints imposed by molecular connectivity.

Consider the row of stems model where a stem is a sequence of polymer chain which traverses the crystalline core of the lamella (Fig. 1*b*). Consider a row of stems perpendicular to the growth face which grows at its end (one is dotted in Fig. 1*b*). A growth face in Fig. 1*b* consists of a parallel array of ends of rows. Each stem is made up of units so that the row consists of a two-dimensional array of units with a reduction in energy ε associated with notional 'bonds' between any adjacent units (intrastem along z and interstem along x, see Fig. 2 and legend). Units are added or removed, with probabilities governed by rate constants which depend on the appropriate Boltzmann factors[5,6]. Molecular continuity is included by a simple additional rule: units are only free to add or subtract at the extremity of the row ($x=1$, Fig. 2*a*)—elsewhere the stems are deemed to be pinned. The ends of pinned stems correspond to folds (or molecular ends). If the row of stems did in fact represent an isolated entity (for example, β sheet) this rule would be the natural consequence of the molecule pleating back and forth within the row. For applications to three-dimensional crystals the rule allows for the increase in probability of stems participating in folds the further back they are from the growth front. This model is sufficient to account for the variations $l\sim\Delta T^{-1}$ and $G\sim e^{-K_g/T\Delta T}$ (either using a rate equation approach[6] or computer simulation[5]).

The following simplified treatment of the row of stems model contains the essence of the argument. It is assumed here that all the stems in the completed row have the same length l and that $l_x\leq l_{x+1}$ where l_x is the length of the x^{th} stem ($x=1$ is at the growing end and $x=2$ corresponds to the stems next to the end, see Fig. 2*a*). Consider firstly the model where $l_2=l$; this restriction is equivalent to limiting the initiation of a new stem to configurations where $l_1=l$ (Fig. 2*b*). The growth rate G' for this model is readily obtained by considering the concentrations $C(i)$ of various $l_1=i$ states, and the rates of conversion between them (as for other sequential processes). State $i=0$ is taken to be equivalent to $i = l$. For example, state $l_1 = l$ can either change to state $l_1 = 1$ (by addition of a new stem with a total probability of $C(l)k^+$ where k^+ is the arrival rate of units to the row) or to state $l_1 = l - 1$ (by removal of a unit from the stem, with a probability of $k_1 C(l)$ where $k_1 = k^+ k = k^+ \mathrm{e}^{-\Delta f/kT}$ is the rate constant for stem reduction). The rate constant for stem removal, (C(1) state to C(l)), k_2, is $k^+ \mathrm{e}^{(\varepsilon - \Delta f)/kT}$. The result is l simultaneous equations; for example, one such is obtained by equating creation and removal rates for state $l_1 = 1$ ($C(l)k^+ + C(2)k_1$ and $C(1)k^+ + C(1)k_2$ respectively). The values of the $C(i)$ (and hence the fluxes) can be obtained by solving these equations. As expected, G' depends essentially linearly on temperature (D. M. Sadler, in preparation)

$$G'/k^+ = (1 - k_1^{(l-l_m)})/D \simeq (l - l_m)\Delta f/kTD \quad (\text{as } l \to l_m) \qquad (3)$$

D depends on k^+, k_1, k_2 and l, is of the order of l^2, and is equal to l^2 in the limit $h \ll kT$. The probability $C(l)$, that $l_1 = l$ for the $l_2 = l$ model, can also be calculated and is of the order l^{-1}.

In the more general case of the row of stems model, the configurations with $l_2 = l$ exist as a minority. All the other configurations are not 'viable', in the sense that at least some units must be removed before the process of initiating and lengthening of stems can proceed indefinitely to give a uniform l value in the body of the 'crystal'. These removal events are interpreted as allowing the removal of folds or loops unfavourable to growth. The barrier term arises from the probablility P that configurations are viable ($l_2 = l$).

The probability that $l_1 = l$ in the row of stems model is equal to $PC(l)$ and can be derived using the approximation[13] that the distribution of stem lengths is the same as in equilibrium (that is, for zero growth, with a value of ΔT adjusted so that $l = l_m$). The state $l_1 = l$ has zero energy for the case $l = l_m$, so that its Boltzmann weighting factor in calculating a partition function is unity. A simple mathematical device for enumerating the weighted sum over all configurations (the partition function) is to consider random walks: each walk corresponds to a configuration (for example, as in Fig. 2c). The weighting is achieved by making the ratio of probabilities for horizontal displacements to vertical ones in the walk equal to k_1^{l-z} where z is the height along the stem. The conditions for random walks apply because this ratio depends only on z and not on the previous history of any particular walk. The probability of an initial vertical displacement is unity (the walk must start somewhere) so the probability of $l - 1$ successive vertical displacements to give $l_1 = l$ leads to

$$PC(l) = \pi_{z=1}^{l-1}\{1/(1 + k^{(l-z)})\} \qquad (4a)$$

This result is closely related to an exponential dependence on l. This is shown by simplifying equation (4a) for two limiting cases, according to the magnitude of the largest of the terms $k^{(l-z)}$.

$k^l \approx 1$ means that all the $k_1^{(l-z)}$ are close to unity and

$$PC(l) \simeq 2^{-l+1} \qquad (4b)$$

$k^l \approx 0$ and large l. In this case the product is converted into a sum by taking logarithms, and the sum is approximated as an integral

$$\ln(PC(l)) \simeq -\int_1^{l-1} \ln(1 + k^m)\,\mathrm{d}m$$

A substitution $u = k^m$ then gives

$$PC(l) \simeq \mathrm{e}^{-\pi kTl/12\varepsilon} \qquad (4c)$$

ε appears in the result because, for $l = l_m$, $\varepsilon = l\Delta f$ (from equation (1)).

G is the product of P and G', which are decreasing and increasing functions of l respectively (see the solid lines in Fig. 3 and equation (2)). G has a maximum at l slightly greater than l_m (dashed line in Fig. 3). Just as for the LH theory[15] this is what is required for a kinetic theory to predict chain folding. The derivation of an l value is often made by averaging over such a growth rate, which implies an ensemble of crystals each of uniform l. A second approach[15,16] allows for fluctuations in thickness within each crystal. For the mathematically analogous case of the row of stems model, the numerical solution of the complete set of rate equations[6] allows for fluctuations, and the average l value obtained is less than would be obtained from averaging over the dashed curve[13]. This is because there is an energetic barrier to fluctuating upwards in stem length which is absent for fluctuating downwards.

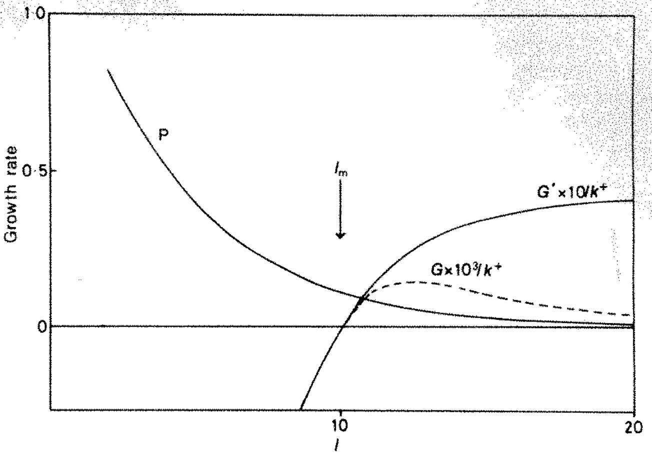


Fig. 3 The growth rate G in numbers of new stems per unit time, dashed line, for the row of stems model (with all the stems in the fully grown rows being of length l). The contributions to this growth rate are P, the probability that a row is viable (see Fig. 2b), and G', the growth rate for viable rows. P and G' were calculated from equations (3) and (4a), with $\Delta T/T_m^0 = 0.05$, $\varepsilon = 3\,kT_m^0$, $h = 6\,kT_m^0$. (See Fig. 1 of Frank and Tosi[15] for the LH model.) G/k^+ and G'/k^+ are normalized growth rates.

The interpretation of the (largely entropic) barrier to growth which exists in the row of stems model is that most stem configurations are tapered; these do not allow the growth front to progress, because in doing so short stems would be engulfed in the body of the crystal and average l values would become $< l_m$. The growth tip constantly explores the available configurations, and when a non-tapered one is reached the front can advance. Equation (4) gives the probability of such situations arising, and shows that it is an exponentially decreasing function of l: hence it is a barrier term e^{-Kl} in equation (2). In applying this to polymer crystals, the rule that changes only occur at the outermost part of the crystal follows from stems elsewhere being pinned, for example by folds.

The exponents in equation (4) can be compared with K_g from experimental plots of $\ln(G)$ against $1/T\Delta T$ (ref. 14), using the accepted estimates of experimental parameters[14], for example, the fold surface free energy which can be used to relate $l(\sim l_m)$ to ΔT^{-1}. The only free parameter is the average number n_u of monomers in a unit, and equating theoretical and observed exponents for equation (4b) gives $n_u \sim 3$ for polyethylene. This probably represents a satisfactory numerical agreement in growth rates considering the simplicity of the model (n_u is by its nature a rather ill-defined quantity; $n_u \simeq 10$ has been suggested[15,9]). Changes in exponent (for example, in going from equation (4b) to (4c)) may relate to observed changes in K_g which have hitherto been associated with regime changes[19,20].

A growth surface roughness which increases with T (ref. 3) is also consistent with a marked increase in G with T when data for the same ΔT are compared[14,26,27].

In conclusion, observation shows that in general re-entrant corners on the crystal growth faces only have a minor influence on the growth rate. Corner (step) creation, 'nucleation', is not therefore the barrier that leads to the distinctive lamella nature of polymer crystals. An alternative kinetic model, based on rough growth surfaces and the kinetic restrictions resulting from molecular connectivity (pinning), appears to be compatible with many experimental observations.

I thank Dr G. H. Gilmer for collaboration on related work which overlaps the present one, and Professor A. Keller and Dr H. D. Keith for assessments of the manuscript.

Note added in proof: It has recently been found that the rates of growth and primary nucleation of long chain paraffins can show minima as a function of temperature[30]. Such an effect suggests an entropy barrier to growth and has been seen in calculations using the row model. The minima correspond to cases where there are significant departures from 'equilibrium' as regards the distribution of l_x, in contrast to the approximation used in the derivation of equation (4a) (Sadler and Gilmer, to be published).

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Namurian uplift in Australia and South America triggered the main Gondwanan glaciation

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The Permo-Carboniferous glacigenic sediment at the base of the Gondwanan basins reflects the culmination[1,2] of the glacial episode that started in Australia and southern South America in the Namurian, after two local episodes in northern South America and adjacent Africa in the Visean and latest Devonian[3] (Fig. 1). Here we show that the start of each episode was associated with orogenic uplift of the bordering regions: northern South America and adjacent Africa in the latest Devonian and Visean, and southern South America and eastern and central Australia in the Namurian. All areas, except southern South America in the Namurian, were at mid- (and not high) latitudes. Thus, we show that whereas the first order control of glaciation was the configuration of the continents and oceans during the closing of Palaeo-Tethys, orogenesis provided a second order control by the accumulation of ice about bordering uplifts during the three glacial episodes.

The late Palaeozoic glaciation of Gondwanaland (Fig. 1) comprised three episodes: (1) in the latest Devonian in northern South America and adjacent Africa[3]; (2) in the Visean in Brazil[3]; and (3) starting in the Namurian in southern South America and eastern and central Australia, extending into the other Gondwanaland continents during the Permo-Carboniferous (300-290 Myr BP) culmination. A refined apparent polar wander path (APWP) for Australia, based on palaeomagnetism[4] (Fig. 2), enables us to determine more accurately than previously the timing of changing palaeolatitudes in Gondwanaland during the late Palaeozoic glaciation. This APWP confirms earlier determinations[5,6] of the pole in the Palaeo-Pacific and its return to Gondwanaland at the end of the Devonian; but, contrary to the premise that glacial centres developed on land in high or polar latitudes[3], we find that the latest Devonian and Visean glacial episodes of South America and the Namurian episode of Australia are mid-latitudinal (30°-60°). During the Permo-Carboniferous culmination, glaciation was polar in Antarctica and Australia but ranged from 60°-20° S in the rest of Gondwanaland. The onset of each glacial episode is therefore not attributable to polar latitude; rather, each glacial episode started during times of orogenic uplift in the bordering regions. For the latest Devonian glacial episode, the uplifts accompanied the latest Devonian deformation of the northern and central Andes[7] and of the Mauritanides[8]; for the Visean episode, the uplifts are indicated by the deposition of piedmont conglomerate in the Ambo Group of Bolivia[9].

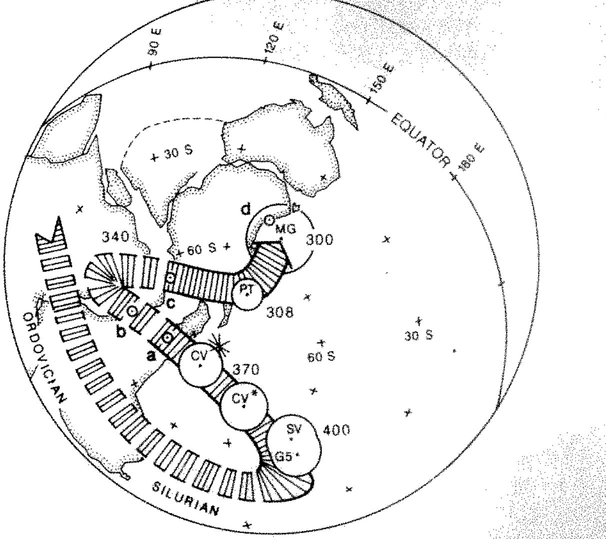

Fig. 1 Revised apparent polar wander path (APWP) for Gondwanaland[32] based on new Devonian poles from Australia (ref. 4 and P. W. Schmidt, personal communication) and reinterpretation of some existing palaeopole positions as likely to be mid-Carboniferous[4]. Palaeomagnetic poles shown are: early Devonian—G5 (ref. 6), SV (Snowy River Volcanics) (P. W. Schmidt, personal communication), taken as 400 Myr; late Devonian—CV and CV* (ref. 4) (370 Myr); late Carboniferous—PT (ref. 34) (308 Myr) and MG (ref. 34) (300 Myr). The Ordovician and Silurian part of the APWP is consistent with Goleby's[6] and Morel and Irving's[5] interpretation of Gondwanan palaeopoles. In the figure *a-d* correspond to the palaeopoles used for the 360 to 300 Myr palaeogeographical maps (Fig. 1).

The earliest Namurian age of the onset of the third glacial episode is indicated by deposits in eastern Australia and southern South America. The oldest record of Palaeozoic ice at or near sea level in Australia is earliest Namurian (333 Myr), as shown directly by the presence of striated stones in the *Cravenoceras*-bearing Kullatine Formation[10] and in the piedmont deposit of the Spion Kop Conglomerate[11]. At the same time, a rapid cooling of sea water is indicated by a sharp fall in the diversity of brachiopods[12], the first appearance of the endemic Gondwana fauna[13], and the last appearance of oolite[14]. The striated stones have been regarded as reflecting an alpine glaciation only[1,15], but we see now that the uplift that shed the Spion Kop Conglomerate was not a narrow mountain chain but instead extended across the 1,000 km-wide Tasman Fold Belt

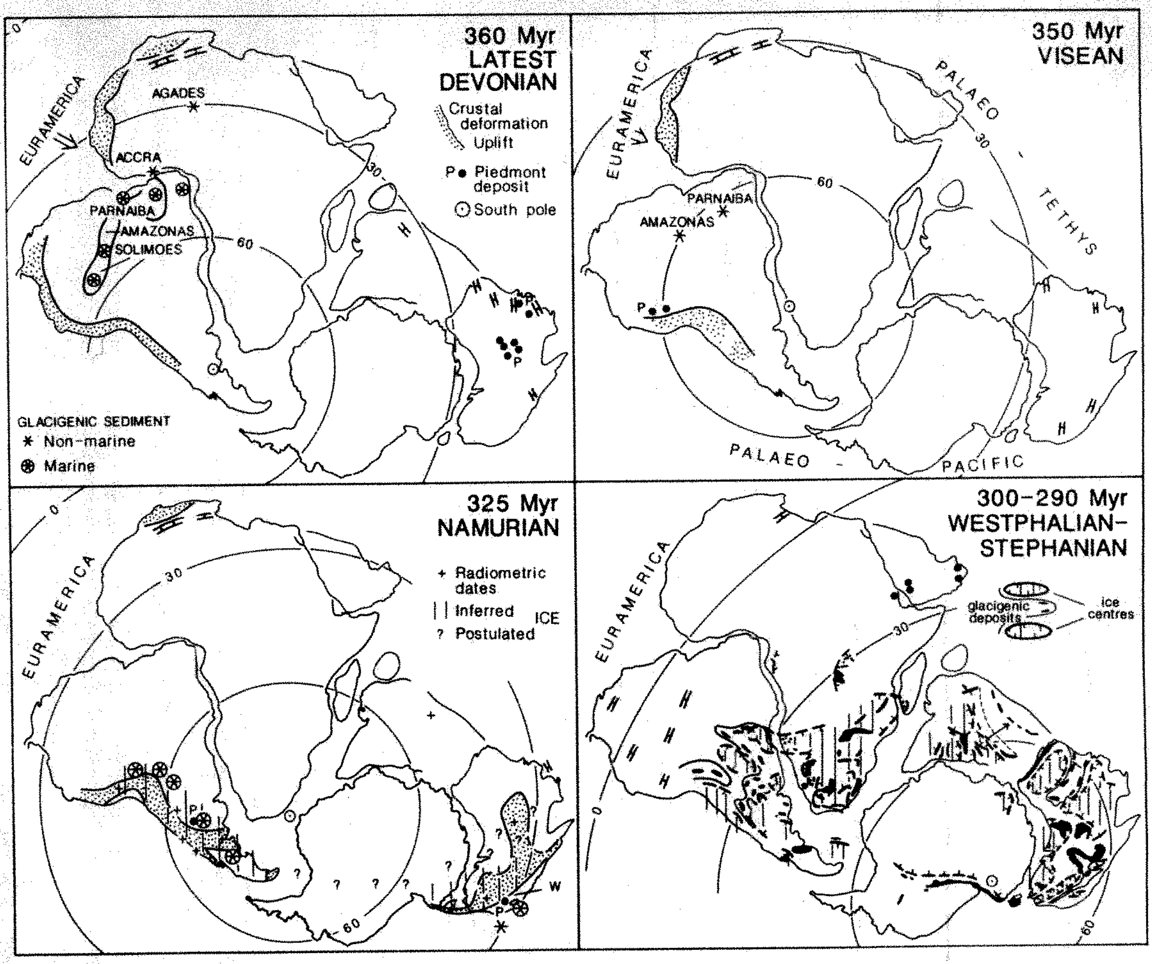

Fig. 2 Palaeogeography of Gondwanaland at 360, 350, 325, and 300-290 Myr, showing latitude (from Fig. 2), metamorphic and plutonic radiometric dates (from Fig. 3), deformation and uplift[7,8,19-21], piedmont deposits[9,11], glacigenic deposits[1,3,11,19,31], inferred and postulated ice and carbonates. Gondwanaland reconstruction[32] with northern margin of Greater India shown palinspastically by removing the Cenozoic rotational underthrusting[33], indicated at 300 Myr by the arrow.

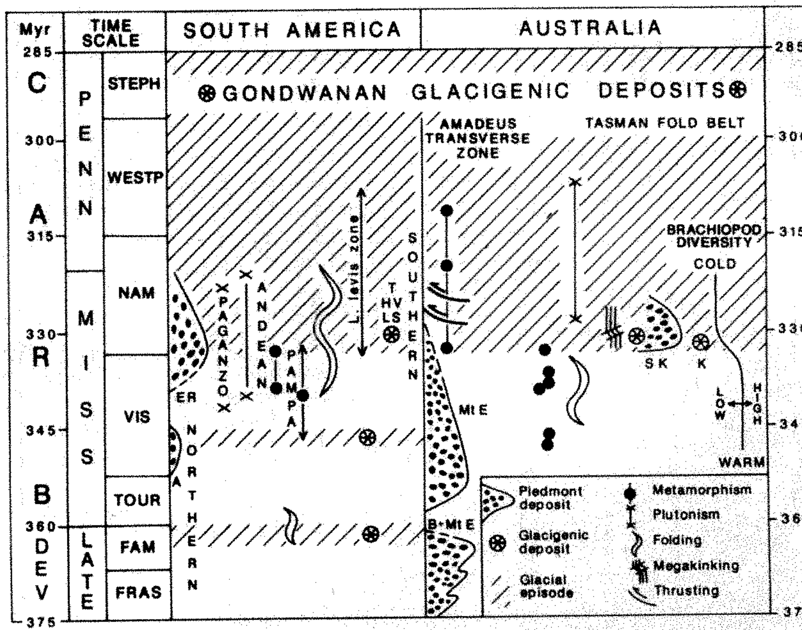

Fig. 3 Timetable[35] of events in South America and Australia during the late Devonian and Carboniferous. Shown for northern South America are the latest Devonian folding and uplift[7], of the same age as that of the Mauritanides[8], both concurrent with glaciation[3], and Visean uplift, indicated by the Ambo Group (A) of Peru[9], concurrent with glaciation in Brazil[3]. Shown for southern South America are the Namurian piedmont El Raton Formation (ER), plutonism in the Paganzo[36] and Andean[21-24] regions, metamorphism in the Andean[21] and Pampa[22-24] regions, folding[21], the range of the *Levipustula levis* zone (ref. 9), and the estimated age of the glacigenic sediments of the Las Salinas (LS), Hoyada Verde (HV), and Tarija (T) Formations (refs 25, 26). Younger glacigenic sediments up to the Westphalian/Stephanian culmination not shown. For Australia are shown the events in (1) the Amadeus Transverse Zone: the piedmont deposits of the Brewer Conglomerate (*B*) of the Amadeus Basin[17] and the Mount Eclipse Sandstone (Mt E) of the Ngalia Basin (ref. 18 and personal information), metamorphism dated by the Rb/Sr ages[37,38] of micas generated during retrogressive metamorphism of Proterozoic rocks, and thrusting [18,37]; and (2) the Tasman Fold Belt: metamorphism[39,40], plutonism (S. E. Shaw, personal communication), folding and megakinking[16,41], the piedmont and glacigenic Spion Kop Conglomerate[11] (SK), the glacigenic Kullatine Formation[10] (K), and the sharp fall in diversity of brachiopods, interpreted as a change from warm to cold[12], which coincides with the first appearance of the Gondwanan fauna[13] and the last appearance of warm-water oolite[14].

and Victoria Land, and through the Amadeus Transverse Zone of central Australia (Fig. 1, 325 Myr). In the Tasman Fold Belt, Visean (340 ± 10 Myr) deformation and metamorphism by east-west (Palaeo-Pacific) shortening was followed in the Namurian by north-south compression that produced megakinks and the intrusion of granitoids, while the Proterozoic basement and Adelaidean and Palaeozoic cover of the intracratonic Amadeus Transverse Zone underwent metamorphism during crustal shortening of 100 to 150 km by overthrusting and the growth of nappes[16]. Precursor uplifts in the latest Devonian produced 3-km thick piedmont deposits in Central Australia[17,18], but the more intense Namurian deformation there lacks equivalent piedmont deposits. The sediments expected to have been shed from the uplifts, then nunataks, were postulated to have been incorporated in the ice and transported to the marine edge of the sheet to be deposited as dropstones, or to have been protected in the ice until its decay in the Stephanian[19]. An alternative possibility is that glacial deposits were reworked into the base of the Permo-Carboniferous basins so that only a few, if any, deposits were preserved.

We have since found that the mid-Carboniferous uplift can be extended to Victoria Land[20] and, more importantly, on the other side of Gondwanaland, to the Palaeo-Pacific margin of South America. Here a major deformation ('Eohercynian Phase', ref. 21) in the Andean region and in the northern Patagonian region of Argentina[22] is dated by syntectonic metamorphism and plutonism[21,23,24], and is accompanied and followed by rapid uplift as shown by the Namurian[25] piedmont El Raton Formation. The glacigenic marine Hoyada Verde Formation of the Calingasta/Uspallata Basin[26], the Las Salinas Formation of the Central Patagonian Basin[26], and the Tarija Formation of Bolivia[9] lie within or below the *Levipustula levis* zone. The overlap of the Mississippian plant *Archeosigillaria converta* in the *L. levis* zone of the Las Salinas Formation[25] indicates that the glacigenic sediments were deposited in the lower or Namurian part of the range of this zone. These events show that South America's development in the Namurian paralleled that of Australia (Fig. 3): orogenic deformation accompanied by uplift and glaciation[27]. That the glaciation was substantial is shown by glacio-eustatic drawdown in Euramerica at the start of cyclothemic deposition[28].

The plate-tectonic control of the configuration of the continents and oceans, in particular the late Palaeozoic closing of Palaeo-Tethys by the joining of Gondwanaland and Euramerica[2,29] and the attendant reduced rate of subduction and lowered concentration of atmospheric CO_2 (ref. 30), is taken as the first order cause of the late Palaeozoic glaciation. Gondwanaland drifted beneath the South Pole in the late Devonian, thus making it susceptible to glaciation, yet widespread glaciation did not start until the Namurian, and then it was mid-latitudinal except in southern South America. What distinguished the Namurian was extensive deformation and uplift in two widely separated parts of Gondwanaland. The correlation in space and time of orogenic uplift with the two preliminary glaciations, and with the Namurian part of the main glaciation, thus leads us to conclude that orogenesis provided the trigger for the late Palaeozoic glaciation.

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Bacteriohopanetetrol from chemical degradation of an oil shale kerogen

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Microbial lipids play an important role in the formation of geologically derived organic matter[1]. Among these lipids the hopanoids have a key function as they show an almost ubiquitous occurrence in sediments and fossil fuels[2]. In this report we have obtained bacteriohopanetetrol, the well-known presumptive precursor of most fossil hopanoids, by catalytic hydrogenolysis of the Messel oil shale kerogen (Eocene, 50 × 10^6 yr). The existence of chemically bonded biohopanoids in an Eocene kerogen supports the idea of incorporation of intact biological structures into high molecular weight organic matter of geological origin. Selective chemical degradation is, therefore, a valuable tool in the structural characterization of polymeric organic matter in the sedimentary environment.

Extensive research has been conducted since the first discovery and identification of geohopanoids[3]. Investigations have concentrated on the stereochemical transformations of this compound class in order to obtain maturity indicators for organic matter[4]. Although the biological precursors of fossil hopanoids were unknown for a long time, their distributions have intensive geochemical application in basin studies or pollution studies of Recent sediments[5,6]. Particular distribution patterns have, in addition, been related to palaeoenvironmental factors such as salinity changes and evaporative processes[7]. It was only in 1973

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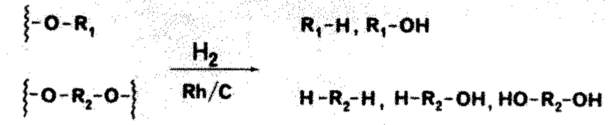

Fig. 1 Diagram showing catalytic hydrogenolysis and its products.

that Förster *et al.*[8] were able to isolate a bacteriohopanetetrol. Rohmer and Ourisson[9] recognized that such a highly oxygenated compound was the potential precursor of the fossil hopanes. Furthermore, its widespread occurrence in bacteria has led to the general assumption that the tetrol serves as a structural equivalent of sterols in membranes of prokaryotic organisms[10,11].

Despite the fact that bacteriohopanetetrol is ubiquitous in bacteria, there have been only a few reports of its presence in geological samples. Rohmer *et al.*[12] observed the coexistence of geohopanoids (hydrocarbons) and their oxygenated precursors, bacteriohopanetetrol and diplopterol, in a Recent mud. The analysis of cyanobacterial mats indicated the presence of a free tetrol[13], and considerable amounts of bacteriohopanetetrol were found in an organic rich soil[14]. However free bacteriohopanetetrol has not been isolated from any pre-Quaternary sample. Because of the presence of the four hydroxy groups, it is highly unlikely that the compound survives oxidative or reductive processes in its free form during diagenesis. Rohmer *et al.*[12] could not detect bacteriohopanetetrol in the soluble low molecular weight fraction of the Messel oil shale, which is known to have undergone only very mild diagenesis.

Our work on the chemical degradation of high molecular weight organic material in sediments (that is, kerogen) has shown that molecular entities of microbial membranes can be incorporated into the matrix of the polymer and thus survive early diagenetic reactions[15,16]. Even labile oxygenated compounds such as lignin-derived phenols could be obtained from kerogen by chemical degradation without loss or destruction of the oxygen containing functional groups[17]. We present evidence that intact bacteriohopanetetrol, incorporated into the polymeric matrix of an oil shale kerogen, can be released by chemical degradation.

The kerogen of the Messel oil shale was degraded by hydrogenolysis using rhodium-on-charcoal as a catalyst. A soluble organic fraction was obtained from the kerogen (organic carbon content = 69%), which was prepared by conventional HCl/HF techniques. This fraction was further separated by chromatography. Among the hydrocarbons, the pentacyclic triterpanes of the hopane type gave the most interesting results. The extractable fraction of the Messel oil shale is known to contain hopanes with carbon numbers not exceeding C_{32} compounds[18]. However degradation of the kerogen revealed extended $17\beta(H),21\beta(H)$-hopanes up to C_{35}. The use of deuterium instead of hydrogen during the degradation experiment gave a series of hopanes with deuterium incorporation into the side chain. On this basis, it was suggested that the hopanes were originally linked via the side chain to the polymer. It was, therefore, highly probable that bacteriohopanetetrol, linked in such a way, was the precursor of the liberated hopanes[19].

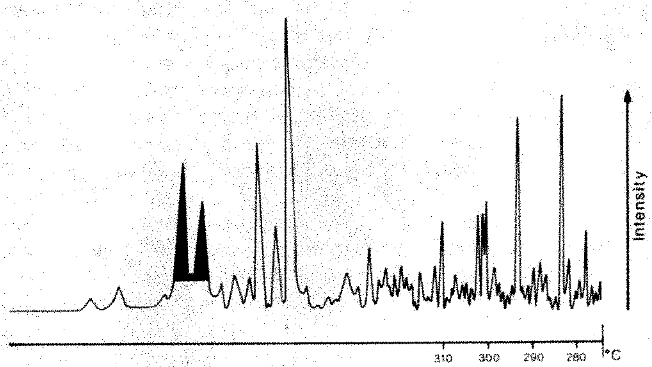

Fig. 2 Gas chromatogram of the polyalcohol fraction (acetates) of the Messel oil shale kerogen hydrogenolysis. The mass spectrum of the earlier eluting of the shaded peaks is shown in Fig. 3. Analytical conditions: 8 m × 0.25 mm SE 54 fused silica capillary column; temperature programme: 200 °-310 °C, 10 °C per min; carrier gas: H_2.

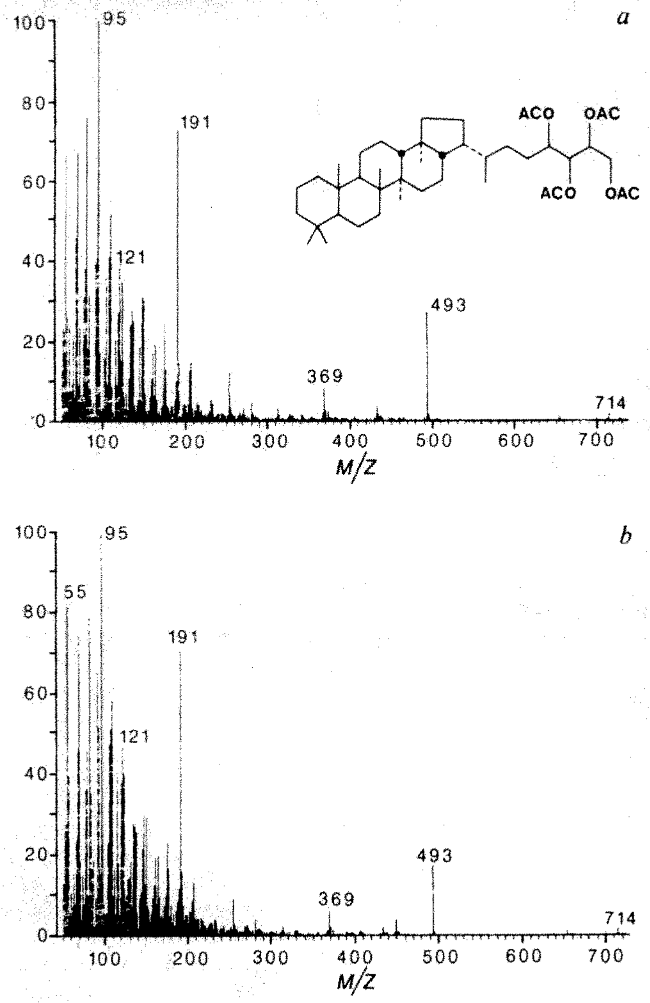

Fig. 3 Mass spectra of bacteriohopanetetrol tetraacetate from *Bacillus acidocaldarius* (*a*) and obtained from chemical degradation of the Messel oil shale kerogen (*b*). Gas chromatography-mass spectrometry, full scan mode, Varian CH7A, ionizing energy 70 eV, source temperature 250 °C; gas chromatography conditions: 30 m × 0.2 mm DB 5 fused silica capillary column; 80 °C, 5 min isothermal; 80 °-320 °C, 15 °C/min.

Catalytic hydrogenolysis is known to cleave ether bonds to give the corresponding alcohols and hydrocarbons (Fig. 1). Ether linked biohopanoids should, thus, give free bacteriohopanetetrol in the alcohol fraction of the kerogen degradation products. In order to avoid secondary reactions, the hydrogenolysis products were acetylated (Py/Ac_2O) immediately after degradation of the kerogen. Several chromatographic steps were performed to isolate the acetylated polyalcohol fraction. Thin layer chromatography (2 mm SiO_2; hexane/ethyl acetate; 3:7 v/v) of the crude acetylated reaction product yielded a fraction with R_F values higher than 0.7. Thin layer chromatography was applied further to this fraction (0.5 mm SiO_2; methylene chloride). The fraction having an R_F value <0.15 was finally purified (0.25 mm SiO_2, thin layer chromatography; hexane/ethyl acetate; 10:7 v/v); the acetates of polyhydroxy compounds ($R_F = 0.5$) were eluted (chloroform/methanol; 1:1 v/v) and analysed by gas chromatography-mass spectrometry.

A partial gas chromatogram of the acetylated alcohols is presented in Fig. 2. Both marked peaks showed identical mass spectra which compare well with the peracetylated bacteriohopanetetrol obtained from *Bacillus acidocaldarius* (Fig. 3). Co-injection on capillary gas chromatography (GC) columns revealed the first eluting peak to have an identical retention time to the biological reference compound with the assigned stereochemistry $22(R)$-$17\beta(H),21\beta(H)$. The later eluting peak did not co-elute with the corresponding $22(S)$ isomer. This was confirmed by coinjection with reference compounds from *Acetobacter aceti* sp *xylinum* which is the only bacterium known at present to contain both the $22(R)$- and $22(S)$-bacteriohopanetetrols[9]. The $22(S)$-isomer elutes first on conventional capillary GC columns.

The discovery of bacteriohopanetetrol in the reaction product of a kerogen hydrogenolysate suggests the existence of intact biological membrane remnants in the high molecular weight fraction of a 50 million yr old oil shale. These membranes are obviously of prokaryotic origin and reflect a significant contribution of such microorganisms to the organic matter of the sediment. In addition the drastic kerogen isolation steps (HCl/HF treatment) have left these membrane constituents obviously unchanged. Future work will show if sediments with a more severe temperature history than the Messel oil shale contain these ether linked bacteriohopanetetrols which can be released by specific degradation reactions. Hydrogenolysis using Rh/C as a catalyst proves its powerful degradative potential by releasing a variety of highly functionalized compounds in structural studies of geological polymeric organic matter.

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A Burgess shale-like fauna from the Lower Cambrian of North Greenland

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The 'Cambrian explosion' refers to the major adaptive radiation of the metazoans during the earliest Phanerozoic, an event that is best known by the abrupt appearance of hard parts near to the Precambrian–Cambrian boundary (~550 Myr BP)[1]. Deciphering this important evolutionary episode depends largely on the study of these skeletal remains[2,3], together with trace fossils that include burrows and trackways[4,5]. Many of these latter fossils evidently represent the activities of soft-bodied organisms, such as worms and lightly sclerotized arthropods, whose exact identity is seldom known, not least because they can be fossilized only in exceptional circumstances[6]. However, documenting the soft-bodied representatives of early metazoan communities cannot be neglected if they accounted for a substantial fraction of these early biotas[7]. Here we report a new soft-bodied fauna from the Lower Cambrian of North Greenland. This assemblage has highly significant similarities with the younger Burgess Shale, and provides additional evidence for the geological longevity of this faunal type and its widespread distribution in deeper waters of Cambrian oceans.

The Buen Formation, from which this new assemblage derives, is a widespread unit outcropping across much of North Greenland. In Peary Land this unit is a siliciclastic shelf sequence[8,9]. A lower sandstone unit records inshore, tidal and high-energy storm-dominated environments, whereas the overlying recessive unit of predominantly mudstones reflects a low-energy, deeper outer-shelf environment deposited close to storm-wave base[10]. The new assemblage is represented by a small collection from a single locality near J. P. Koch Fjord, western Peary Land (Fig. 1), where a small fault-bounded block exposes fissile black shales near the top of the formation.

The Buen Formation is dated as mid-to-late early Cambrian, and the lower part of the overlying Brønlund Fjord Group also yields late Lower Cambrian faunas[9,11]. In terms of north American Lower Cambrian biostratigraphy[12] a position within the lower part of the *Bonnia–Olenellus* Zone is most likely, but an even older age within the underlying *Nevadella* Zone is conceivable[11]. The occurrence in the assemblage of an olenellacean trilobite broadly supports the postulated age, but the shelly fauna lacks other trilobites and is restricted otherwise to

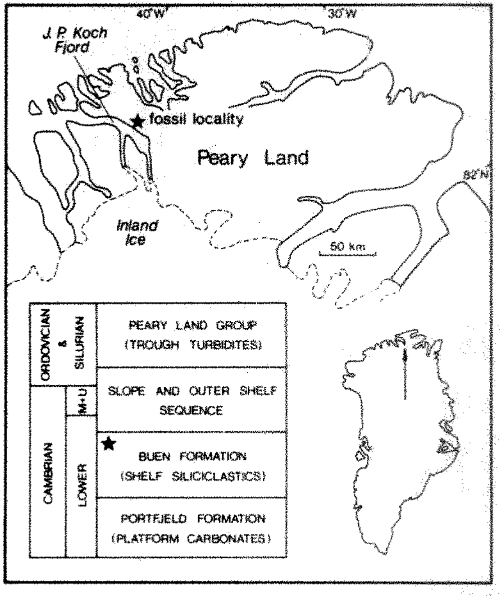
40°W
30°W
J. P. Koch Fjord
fossil locality
Peary Land
82°N
Inland Ice
50 km
ORDOVICIAN & SILURIAN
CAMBRIAN
M+U
LOWER
PEARY LAND GROUP (TROUGH TURBIDITES)
SLOPE AND OUTER SHELF SEQUENCE
BUEN FORMATION (SHELF SILICICLASTICS)
PORTFJELD FORMATION (PLATFORM CARBONATES)

Fig. 1 Locality of new soft-bodied fauna in Peary Land, northern Greenland (and arrow on map lower right inset). Inset column (lower left) shows position of Buen Formation in the regional stratigraphy. The fauna occurs near the top of the Buen Formation, in beds equivalent to either the *Nevadella* or *Bonnia–Olenellus* zones (ref. 11).

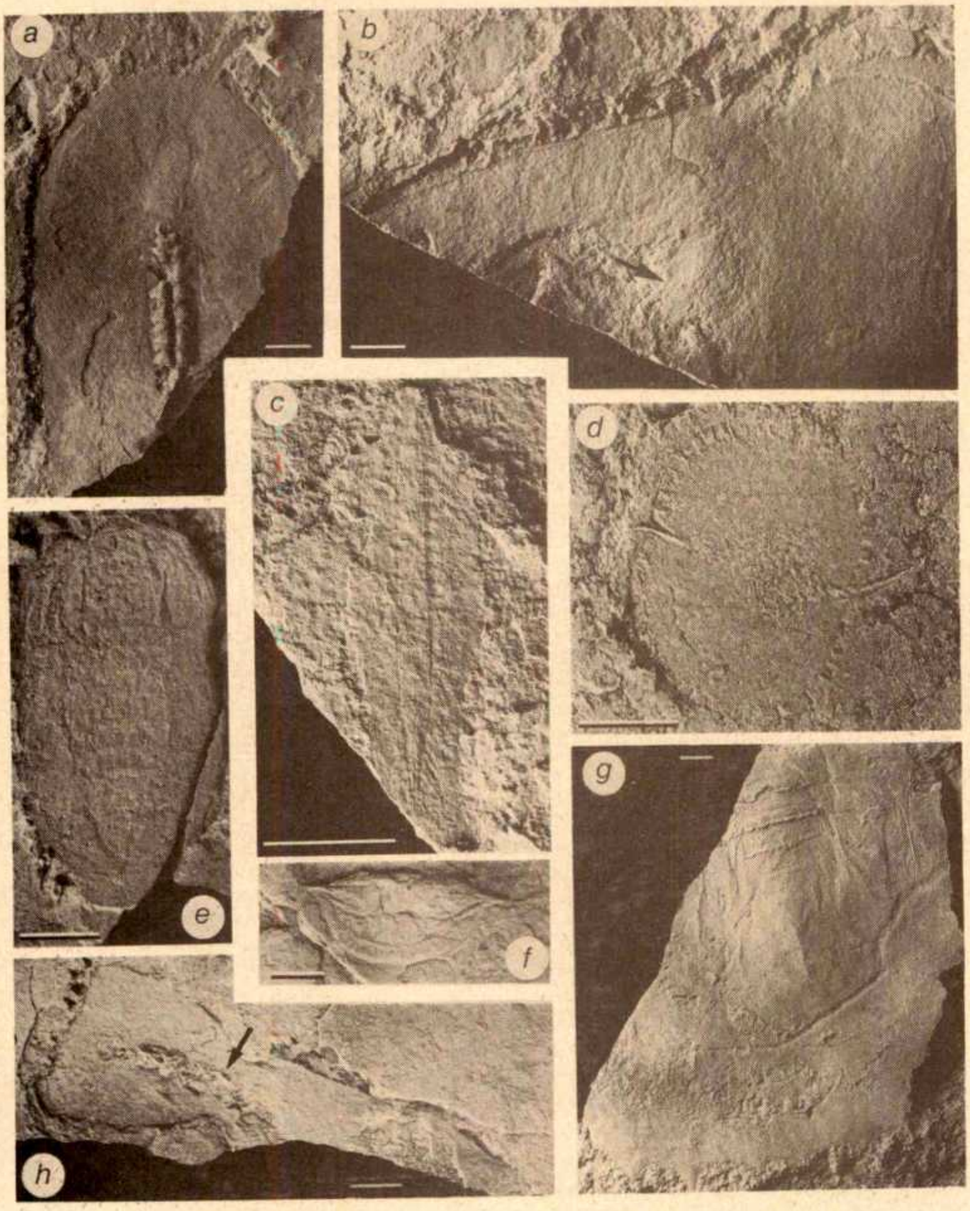

Fig. 2 Arthropods from the Buen Formation, Peary Land, northern Greenland. *a*, Anterior half showing oval headshield and segmented thorax. Note anterior antennae (arrowed) and prominent median gut-fill (Geological Museum, Copenhagen specimen no. MGUH 17512). *b*, Incomplete specimen with bivalved carapace, protruding segmented abdomen, and possible appendages (arrowed) (MGUH 17513). *c*, Posterior section of *Leanchoilia*-like arthropod with segmented thorax and spinose telson (MGUH 17514). *d*, Bradoriid carapace with prominent spines (MGUH 17515). *e*, Isopygous form with cephalon and posterior shield separated by six thoracic segments (MGUH 17517). *f*, Carapace of *Isoxys* (MGUH 17518). *g*, Posterior of large arthropod with marginal spines (MGUH 17516). *h*, Posterior section showing abdomen terminating in a tail fan, with gut contents (arrowed) (MGUH 17519). All specimens from Geological Survey of Greenland sample 319571, deposited in the type collection, Geological Museum, Copenhagen (MGUH). Scale bars, 5 mm.

inarticulate brachiopods. Siliceous sponge spicules are very abundant, and articulated material of the demosponge *Choia*[13] is consistent with the conditions of exceptional preservation documented here.

The soft-bodied component of this locality is dominated by non-trilobitic arthropods that lack mineralized skeletons. The most abundant form (Fig. 2*d*) is represented by a semi-circular bivalved carapace (7.5–14 mm long), with short anterior and posterior spines projecting upwards from the hinge line at a steep angle. This arthropod appears to be most similar to Lower Cambrian bradoriids such as *Sunella*[14,15], but it differs in being larger and having spines of equal length. Somewhat similar to this presumed bradoriid is the larger *Isoxys* (Fig. 2*f*), with longer anterior and posterior spines that project without pronounced curvature.

The largest of these arthropods, growing to at least 130 mm long, is represented by 10 specimens. The smooth cephalon was semi-circular in outline, and bore a pair of elongate segmented antennae that arose adjacent to a poorly defined structure that appears to represent a ventral plate or hypostome (Fig. 2*a*). The thoracic region was divided into approximately 12–13 narrow tergites, and the posterior shield seems to have been broadly similar to the anterior cephalon. Incomplete specimens, questionably assigned to this form, show a posterior shield with at least four pairs of marginal spines (Fig. 2*g*). Information on the internal anatomy is restricted to a gut trace, that includes a prominent nodular infill (Fig. 2*a*) with impressed striations that may represent enteric musculature. Overall it appears to be most similar to *Tegopelte*, an aberrant trilobite from the Burgess Shale that grew to over 250 mm in length[16]. One obvious difference is that *Tegopelte* had three thoracic tergites, but these probably represent a fused complement of at least 10 somites.

The remaining arthropods represent at least three taxa, but are all rare. One type has roughly equal-sized (isopygous) cephalon and posterior shield that are separated by six thoracic tergites (Fig. 2*e*). An axial region defines a longitudinal trilobation along much of the body. This arthropod has a generalized resemblance to a number of Cambrian forms; for example, *Livia plana* from the Lower Cambrian of northern Poland[17]. Another distinctive arthropod bore a semi-circular bivalved carapace, from which appendages apparently protruded (Fig. 2*b*). Poor preservation precludes confident identification, but they may have included filamentous gills and possibly ambulatory appendages. Projecting from the carapace was a relatively slender abdomen, composed of at least six somites, and lacking appendages. Similarities may exist with such Burgess shale arthropods as *Canadaspis perfecta*[18] and *Waptia fieldensis*[19,20]. An incomplete specimen, possibly distinct from the bivalved arthropod, has a prominent tail fan composed of a telson flanked by flattened plates (Fig. 2*h*). A prominent gut trace shows that the anus was located on the telson. Another fragmentary specimen is represented by a series of about 20 thoracic segments, that terminate in an elongate telson bearing a prominent series of at least 8 spines on either side (Fig. 2*c*). A spiny telson is particularly characteristic of the arthropod *Leanchoilia*, best known from the Burgess Shale[21], as well as other middle Cambrian horizons[22,23].

This new soft-bodied fauna is important for several reasons. First, it increases our knowledge of Cambrian diversity and confirms the importance of arthropods, especially lightly sclerotized taxa. In this assemblage, and better known examples such as the middle Cambrian Burgess Shale, these arthropods far outnumbered the better-known trilobites in terms of both numbers of individuals and biomass[7].

Second, in general terms this fauna has striking similarities with a number of soft-bodied Cambrian biotas such as the well-known Burgess Shale[7,24,25], immediately adjacent localities[22], and additional horizons from the United States[23,26-31] and China[32-34]. Together, they reveal a broadly comparable Cambrian biota with a distinct identity. These exceptional faunas share the significant character of having been deposited in deeper water shales, on the margin of the outer detrital belt (or its equivalent) and facing the open ocean. This soft-bodied fauna in north Greenland adds to our knowledge of the circum-Laurentian distribution of Burgess Shale-like faunas[35]. Moreover, similarities between the soft-bodied fauna of South China[32-34] and those encircling the Laurentian craton, suggest that migration by the ocean floor across a 'proto-Pacific' was possible, in a manner comparable to that inferred for Upper Cambrian deep-water trilobites[36].

Third, there is evidence that a number of the Burgess Shale-type genera had unusually long stratigraphic durations. This gives these soft-bodied assemblages a rather conservative aspect in evolutionary terms, and finds a significant parallel in the generic longevity of other deep-water biotas[37]. The occurrence reported here is a particularly important addition, because it and the Chinese[32-34] occurrences are amongst the earliest known. The extension of stratigraphic ranges of at least some Burgess Shale-like taxa back into the early Cambrian also suggests that they were an integral part of the initial diversification of metazoans.

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Ketamine–xylazine anaesthesia blocks consolidation of ocular dominance changes in kitten visual cortex

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In the visual cortex of mammals, response properties of single neurons can be changed by restricted visual experience during early postnatal development[1]. Covering one eye for four to eight hours when kittens are at the peak of the sensitive period is sufficient to weaken the influence of the occluded eye on cortical neurons resulting in a noticeable shift of ocular dominance towards the open eye[2–5]. The underlying changes in synaptic connections do not occur so readily when a kitten is anaesthetized and paralysed[6–8]. We report here that an ocular dominance shift is prevented in alert kittens that receive repeated brief monocular exposures when these are followed by ketamine–xylazine anaesthesia. This retrograde effect on cortical plasticity suggests that the process by which synaptic activity is converted into structural changes has been disturbed.

Kittens were kept in a dark room from 3.5 weeks of age. On every other day they were exposed to a high-contrast visual environment for 20 min with one eye occluded by a black contact lens. This was repeated 15 times, resulting in 5 h total monocular experience. After each 20-min exposure the kittens were returned to the dark. Control animals were given sham injections of NaCl solution (0.9%, 2 ml per kg body weight). The experimental animals received an intramuscular injection of ketamine-hydrochloride (Ketanest 1%, 25 mg kg^{-1}) supplemented with xylazine (Rompun 5%, 2 mg kg^{-1}). Ketamine–xylazine is a standard veterinary combination anaesthesia used for its rapid onset and minimal effects on blood pressure. With our dosage anaesthesia lasted for about one hour (mean 74 ± 12 min). All kittens were weighed regularly, and no difference in their rate of physical development was seen from their body weight.

Two days after the last exposure session a double-blind recording experiment in striate cortex was performed. Standard physiological techniques were used[9,10]. Animals were anaesthetized with halothane (0.4%) and N_2O/O_2 (70% : 30%). Individual results are shown in Table 1. The ocular dominance (OD) distributions of two control (R1, R3) and three experimental animals (R2, R4, R5) are shown in Fig. 1*a* and *b*, respectively. Kittens R1 and R3 showed a clear dominance of the exposed eye, but the three kittens with regular post-exposure anaesthesia had an even OD distribution.

To ensure that the total exposure time of 5 h had not been too short, we performed another series of experiments in which we gave 30 exposures of 20 min duration to one eye on every day, the total exposure time amounting to 10 h. Again, recording

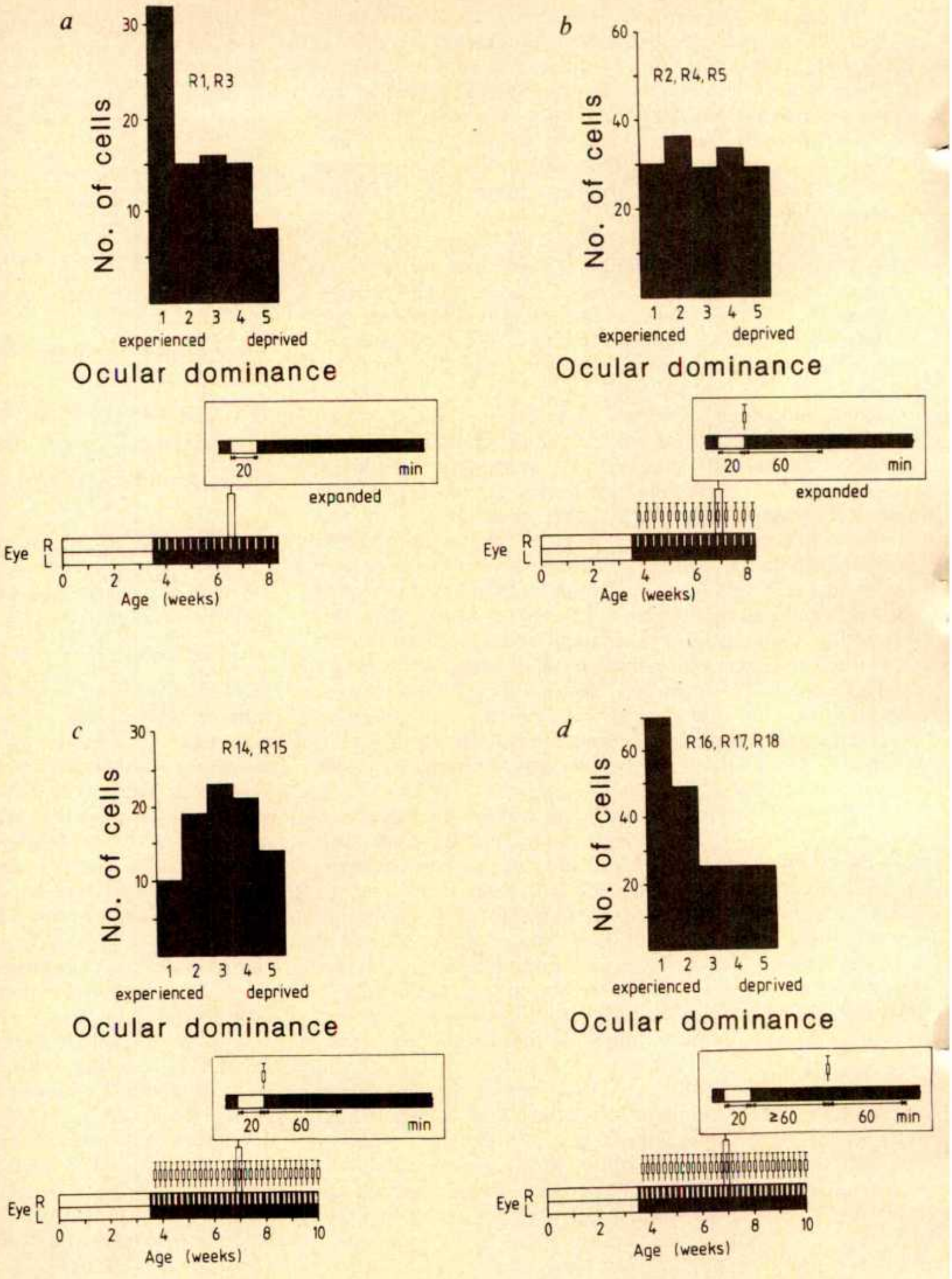

Fig. 1 The ocular dominance distributions of single neurons in visual cortex (area 17) of both cerebral hemispheres from four different groups of kittens. The ocular dominance (OD) of a cell indicates the relative degree of excitability from the two eyes by visual stimulation and is grouped into five categories: class 1(5), only driven through right (left) eye; class 2(4), larger response when stimulated through right (left) eye; class 3, about equally driven through either eye. OD ratings were first determined by hand and were then confirmed quantitatively from PSTHs averaged in at least four, usually eight directions of a moving light bar. Below each histogram the experimental history of the group is shown. Open and filled blocks correspond to light and dark rearing respectively. Three groups of animals (*b*, *c*, *d*) were given intramuscular injections of ketamine-xylazine after each visual exposure as indicated by the syringe symbols. The precise time of anaesthesia is shown in the inserts on an expanded time scale. All receptive fields were within the central 10° of the visual field. No difference was found between data collected from the two cerebral hemispheres. *a*, Control kittens with 5 h total monocular experience given for 20 min every other day, 15 times. Animals were alert and lightly restrained during exposure for continuous stimulation in a high-contrast visual environment of 170 cd m^{-2} mean luminance. *b*, Same exposure protocol as in *a*, but here the kittens were anaesthetized immediately after each exposure. Ocular dominance was not shifted in these animals. *c*, Same protocol as *b*, but total monocular exposure was extended to 10 h. Exposure was given every day. *d*, Same protocol as *c*, but kittens were given a break of one (R16) or six hours (R17, R18) in total darkness after each exposure session before receiving anaesthesia. The shift of ocular dominance in these kittens indicates that retrogade duration of impairment is probably <1 h.

was done blind and all ratings were confirmed quantitatively from peri-stimulus-time histograms (PSTHs). Two kittens (R14 and R15) received the anaesthetic immediately after each exposure. Again their OD distribution showed no signs of a shift towards the exposed eye (Fig. 1*c*).

As further controls, three kittens (R16, R17, R18) with the same exposure schedule received the anaesthetic only after a delay of 1 or 6 h spent in the dark. All three animals showed an OD shift in striate cortex (Fig. 1*d*). This result demonstrates most clearly that anaesthesia did not have an effect on the physical state or on the rate of development of the animals. Moreover, it excludes an anterograde effect of anaesthesia and shows that the retrogade impairment of binocular plasticity lasts not more than an hour. This can also be demonstrated by extending the duration of exposure sessions to one hour (Fig. 2). If in this case anaesthesia is given immediately after each exposure, an OD shift is not prevented (Fig. 2*a*). The shift is as clear as in two control cats that received only sham injections of NaCl (Fig. 2*b*).

The results of this study show that a short period exists during which ketamine-xylazine anaesthesia can retrogradely interfere with the plasticity of binocular connections in the visual cortex of young kittens. The synaptic changes that are normally triggered by neuronal activity[9,11] are prevented or disrupted. The period is reminiscent of what has been termed a 'consolidation period' by others[12]. Our results indicate, however, that consolidation occurs much more rapidly than had been assumed in other studies, which tested the effects of delay after a single 24-h monocular exposure[3,4]. Clearly, in these experiments initial consolidation had already taken place during exposure.

It is of particular interest whether the retrogade impairment of cortical plasticity is specific for ketamine-xylazine. Preliminary experiments in two kittens (U. Egert and J.P.R., in preparation) indicate that the effect cannot be induced by

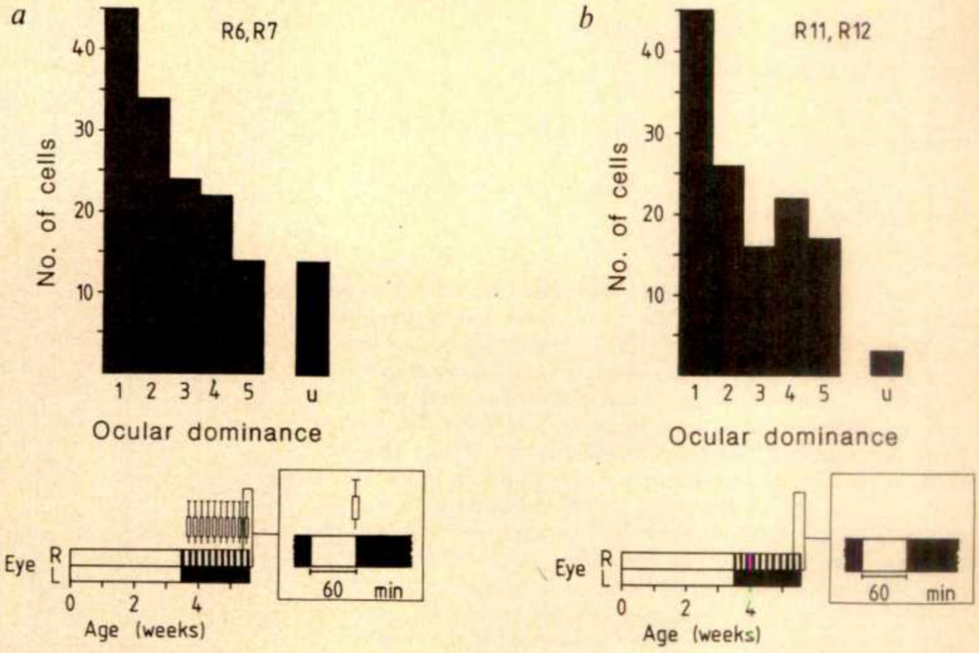

Fig. 2 Comparisons of ocular dominance distributions from (*a*) two kittens that were anaesthetized with ketamine-xylazine immediately after each of ten monocular exposures with those from (*b*) two control animals that received only sham injections of saline. In all cases exposure lasted for 60 min instead of 20 min as in Fig. 1. All animals show a shift of ocular dominance, which supports the conclusion that the duration of a 'consolidation period' is<1 h. Columns marked 'u' show visually unresponsive neurons.

Table 1 Individual results of single unit recording from striate cortex in 14 kittens

Group no.	Animal no.	No. of units analysed	No. of unresponsive units	No. of OD determined in hemisphere contra to deprived eye	ipsi to deprived eye	No. of units in each ocular dominance group n_1	n_2	n_3	n_4	n_5	Weighted OD index $\left(I=\frac{n_1+0.5n_2}{n_5+0.5n_4}\right)$
1*a*	R1	51	15	30	6	13	7	7	5	4	2.5
	R3	70	20	25	25	19	8	9	10	4	2.6
1*b*	R2	57	17	20	20	8	9	4	8	11	0.8
	R4	65	9	34	22	8	14	15	14	5	1.25
	R5	83	20	25	36	14	13	10	11	13	1.1
1*c*	R14	29	6	22	1	4	2	7	5	5	0.7
	R15	82	16	31	33	6	17	16	16	9	0.9
1*d*	R16	74	11	29	30	28	17	7	4	3	7.3
	R17	81	7	35	36	23	16	10	12	10	1.9
	R18	72	7	24	40	19	16	8	9	12	1.6
2*a*	R6	75	7	33	34	17	18	11	13	8	1.8
	R7	80	7	35	37	28	16	13	9	6	3.4
2*b*	R11	69	1	35	33	24	13	8	12	11	1.8
	R12	61	2	30	28	21	13	8	10	6	2.5

The group numbers correspond to the Figure in which data and experimental protocol are shown. The non-parametric Mann–Whitney *u*-test was used to compare the experimental and control groups. If the weighted OD indices of the animals in control groups 1*a* and 1*d* are tested against the animals in experimental groups 1*b* and 1*c*, a significant difference of $P<0.005$ is obtained. Comparison of 2*a*/2*b* with 1*b*/1*c* yields $P<0.01$.

xylazine alone, which is an α-adrenoceptor agonist[13,14]. Ketamine, on the other hand, is known to interfere with memory processes, and its psychotomimetic effects have long been known[15,16]. Recently it has been found to be a blocker of NMDA-receptors for glutamate[15,17,18], blockage of which prevents LTP in hippocampal slices[19,20] and spatial discrimination learning[21].

Clearly, further experiments are needed to determine the pharmacological mechanisms of synaptic consolidation. The important aspect of the present findings is that they demonstrate the existence of a consolidation period for visual cortical plasticity. Consolidation effects are well-established in the psychology of learning[22,23] and our results suggest that the same processes may be involved in visual plasticity as in long-term memory.

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Long-distance intraretinal connections in birds

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Electrophysiological experiments have shown in both birds and mammals that remote parts of the retina, several millimetres apart, interact at the retinal level[1,2]. The anatomical basis of this is poorly understood, although in mammals some cells in the ganglion cell layer have axons that terminate in the inner plexiform layer several millimetres from the cell body[3,4]. In birds, the longest previously reported intraretinal connections were from amacrine cells, extending only a few hundred microns[5]. But we here describe very long connections that span almost the entire extent of the retina in chicks and chick embryos. The parent cell bodies are in the inner nuclear layer of the ventral half of the retina, and they project in topographical order onto the dorsal half. They do not project to the brain. They may be involved in selective switching of attention between the upper and lower parts of the visual field, at an unprecedentedly early stage of visual processing.

We have demonstrated this projection by retrograde transport of the intensely fluorescent carbocyanine dye 1'1-dioctadecyl-3,3,3',3',tetramethylindocarbocyanine perchlorate (DiI), which is known to be transported both anterogradely and retrogradely[6,7]. The dye was inserted into the retinas of anaesthetized embryos and newly hatched chicks (see Fig. 1 legend). It hardly diffused at all, as is shown by the fact that the only labelled optic fibres were restricted to a narrow band 0.1–0.5 mm wide, running through the site of dye placement to the optic nerve-head, and in the opposite direction towards the ganglion and displaced ganglion cell bodies of origin, which were labelled directly along it.

Remote from this stream of labelled cells, other retinal neurons that we shall call 'proprioretinal cells' were labelled in the inner half of the inner nuclear layer. These cells were mostly of two types, equally numerous (Figs 1–3). Type I was the larger, 15–20 μm in diameter. It lay close to the inner plexiform layer and resembled Cajal's 'stratified amacrine cells of the first level'[8], displaying several labelled dendrites which could often be traced for more than 100 μm in the most external level of the inner plexiform layer (Cajal's lamina I; ref. 8). Type II was only slightly smaller, about 15 μm in diameter. It lay deeper in the inner nuclear layer and appeared pear-shaped, with a single thick process extending from the cell body to lamina I of the inner plexiform layer where it could sometimes be seen to branch, although the dendrites could never be followed for more than a few microns; it resembled Cajal's 'association amacrine cells'[8]. From both types labelled axons ran in the outer part of the inner plexiform layer directly towards the dye placement site, where they could no longer be traced because of the high background. Occasionally they appeared to yield terminal arbors just as they entered the high background region.

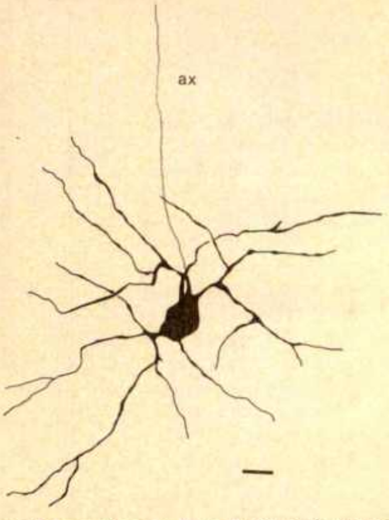

Fig. 2 Tracing of a proprioretinal type I neuron in the ventro-nasal part of a retinal flat-mount from a 4-day-old chick labelled with a fleck of DiI in the dorso-nasal retina; ax, axon. Nasal is right and dorsal is up; scale bar, 10 μm.

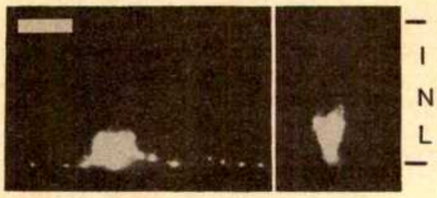

Fig. 3 Type I (left) and type II (right) proprioretinal cells from a 4-day-old chick viewed in a transverse section of the retina to show the differences in their morphology, and in their depth in the inner nuclear layer. Such sections were prepared by removing flat-mounted retinas from the slide and re-sectioning them in a cryostat at 40 μm, after immersion in 30% buffered sucrose. INL, inner nuclear layer; scale bar, 20 μm.

Experiments on embryos of different ages indicated that the proprioretinal projection was detectable on embryonic day 11 (E11) and was fully developed 2–3 days later.

We have studied the topography of the projection in 39 retinas, in 14 of which the distribution of labelled cells was charted quantitatively using a microscope linked to a computer. The position of the dye-insertion was systematically varied so as to test all parts of the retina except the most central region, which was inaccessible. The results are summarized in Fig. 4. The labelled proprioretinal cells were found exclusively in the ventral

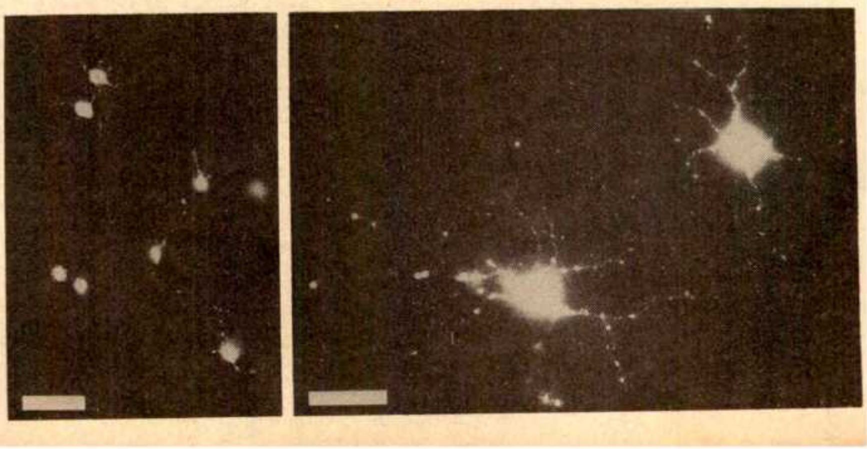

Fig. 1 Proprioretinal cells, viewed in a retinal flat-mount from a 3-day-old chick. Left, 5 type I cells (upper and right) and 2 type II cells (lower left). Right, 2 type I cells at higher magnification. Scale bars: left, 75 μm; right, 30 μm. In all experiments a fleck of DiI was inserted into the right retina through a small cut in the sclera and choroid. After 2–9 days, the birds were killed and the labelled eye removed. The cornea was excised and the eye immersed in 10% formalin in cacodylate buffer (0.1 M, *p*H 7.2) for 3 days at 4 °C. The retina was then flat-mounted, with the vitreal surface up, in an aqueous solution of 90% glycerol and 2% propyl gallate, a coverslip was applied, and the retina viewed with a fluorescent microscope using filters appropriate for rhodamine.

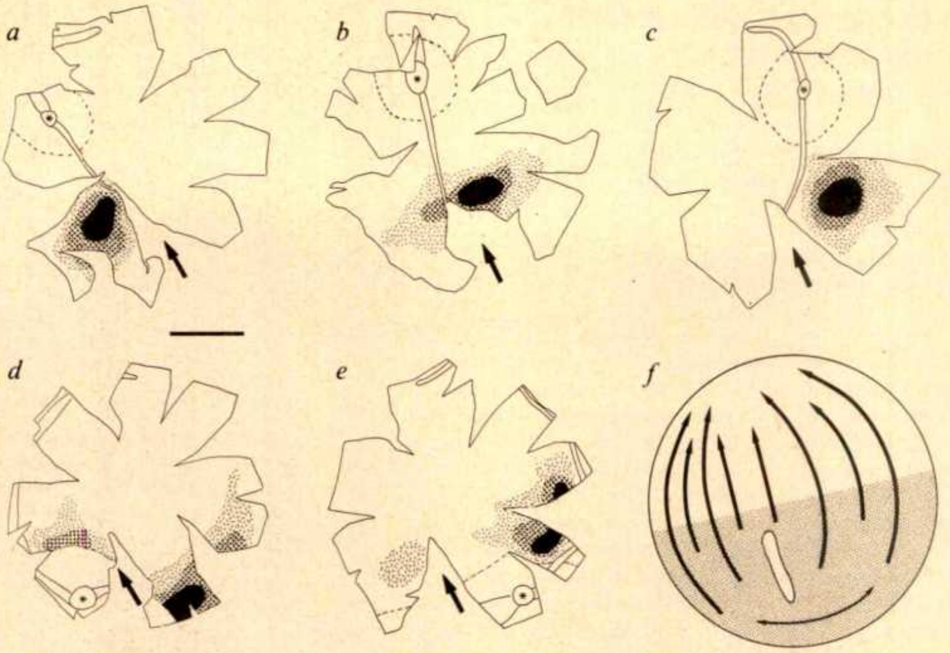

Fig. 4 The topographical arrangement of the proprioretinal projection viewed in flat-mounts. *a–e*, Distribution of the labelled neurons in five 4-day-old chicks following the placement of DiI (asterisks) in different parts of the right retina. The interrupted lines circumscribe the region immediately around the dye fleck where many cells are labelled in all layers. The double line stretching from the dye fleck towards and away from the optic nerve-head (elongated in birds—see *f*) denotes the band of labelled optic axons; the stream of labelled ganglion and displaced ganglion cells lies along this band distal to the dye fleck. Arrows, optic fissure, which allows proper orientation of the retina. The density of labelled proprioretinal cells is as shown: black, 31–80 cells mm^{-2}; heavy stippling, 16–30 cells mm^{-2}; light stippling, 6–15 cells per mm^{-2}. Scale bar, 5 mm. *f*, Summary of the topography. The proprioretinal cells were found exclusively in the stippled region. They project as indicated by the arrows. The cigar-shaped profile represents the optic nerve-head. In all diagrams, nasal is right and dorsal is up.

half of the retina. From here they project both to the dorsal retina and within the ventral half. The former projection shows a clear topographical organization. The ventro-nasal sector proects to the dorso-nasal, the mid-ventral to the mid-dorsal, the ventro-temporal to the dorso-temporal. In addition, the more ventral parts of the ventral hemiretina appear to project the more dorsally in the dorsal half (Fig. 4*f*). It was difficult to define the projection pattern within the ventral region, but many proprioretinal cells in each of the ventral quadrants have axons which pass beyond the optic fissure into the adjacent one (Fig. 4*d*,*e*).

The maximal density of labelled proprioretinal cells was typically 80 mm^{-2} and the area of the ventral hemiretina was about 90 mm^2, which suggests there were not less than 7,000 of them n each retina.

The fact that the proprioretinal cells had labelled axons running directly to the dye fleck and not towards the optic nerve-head suggested that they did not project to the brain, but further experiments were needed to check this point. They might conceivably have had an unlabelled axonal branch running to the optic nerve-head. Or, less likely, the axon destined for the nerve-head might have followed an indirect trajectory through the site of dye placement. Both these possibilities were tested n two series of double labelling experiments using DiI and Fast Blue[9] as complementary dyes.

For technical reasons we used embryos, but at an age when the optic and proprioretinal projections are fully developed. In one series we inserted a fleck of DiI in the dorso-temporal retina at E13–E14, injected the contralateral mid-brain and thalamus wth Fast Blue (2% in water, several injections, total 1.6 μl) at E14, and killed the embryo at E16. In the other series we first injected the Fast Blue in the brain at E12, inserted the fleck of DiI in the retina at E14, and killed the embryo at E16. Nine embryos of the first series and three of the second were well labelled with both dyes.

In frozen sections through the mid-brain and thalamus there was a heavy deposit of Fast Blue in all the primary optic centres, including the ectomamillary nucleus and optic tectum. This led to most retinal ganglion cells being retrogradely labelled. In the inner nuclear layer, blue-labelled displaced ganglion cells[10,11] and red-labelled proprioretinal cells occurred side by side, but none was double-labelled, indicating that none of the proprioretinal cells projects to the brain.

In conclusion, we have shown that long proprioretinal projections occur, and that they originate exclusively in the ventral retina, which represents the upper part of the visual field. A similar bias towards the ventral retina is shown in chicks by the centrifugal fibres from the brain (ref. 7, unpublished). We therefore speculate that the intraretinal connections may be involved in a system for switching attention between the upper and lower halves of the visual field under partial centrifugal control.

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The v-*myc* oncogene is sufficient to induce growth transformation of chick neuroretina cells

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A number of studies have shown that full transformation of non-established rodent fibroblasts can be efficiently achieved *in vitro* by the concerted action of two oncogenes belonging to different complementation groups (reviewed in ref. 1). Extension of the two-genes carcinogenesis model to other differentiated cell types, presumably endowed with different controls of growth, is desirable for a better understanding of questions such as the host cell selectivity of oncogene action. A recent report[2] claimed that cooperation between two oncogenes, v-*myc* and v-*mil*, is required to achieve transformation of chicken embryo neuroretina cells, which are characterized by a limited growth capacity in monolayer culture[3]. Here we present evidence that the v-*myc* oncogene alone is sufficient to induce growth transformation of glial and neuronal precursor cell types from chick neuroretina. We also report that induction of transformation by v-*myc* is accompanied by faithful preservation of some of the differentiated functions of the chick cells.

Dissociated neuroretina (NR) cells from 7-day chicken embryos, consist primarily of replicating neuroepithelial cells belonging to both the glial and neuronal lineages and post-mitotic neurons. As the proliferation state of a cell population may affect the efficiency of infection and subsequent spread of virus by re-infection, optimized culture conditions were established (see Fig. 1 legend), under which uninfected NR cells could be successfully cultivated for as many as 10–15 population doublings before cells completely ceased growing. To assess whether the v-*myc*-containing viruses MC29 and MH2 (which also carries the v-*mil* oncogene) can transform neural cells *in vitro*, NR cells were infected with MC29, MH2, PR-A (which carries the v-*src* oncogene) and the non-transforming Rous-associated virus 1 (RAV-1)[4]. Cultures were then passaged twice and assayed for the expression of transformation-associated traits. Third-passage, morphologically transformed NR cells, subcultured at low density, showed reduced doubling times and increased rates of DNA synthesis as compared with uninfected or RAV-1-infected cells of the same age *in vitro* (Fig. 1*a*). To determine the percentage of infected NR cells producing transforming virus at the same passage, RSV, MC29 and MH2-infected cells were treated with mitomicin C and tested for their ability to induce secondary transformed foci after replating on chicken embryo fibroblasts. The infectious centre tests revealed that at this stage the majority (>80%) of NR cells in culture produced RSV or MH2, whereas in the case of MC29 only 30% of cells were virus producers. The latter value may explain the kinetics in Fig. 1*a*. NR cells uniformly infected by MC29 and MH2 shed high numbers of cells into the medium after reaching confluence, maintained a sustained growth rate as shown by ^{3}H-thymidine incorporation and cells could be efficiently grown *in vitro* for up to 50 population doublings. To determine whether virus-infected cells had acquired anchorage-independent growth properties, fifth-passage infected infected cells were seeded in soft-agar and colonies counted two weeks later. Table 1 shows that all viruses were capable of inducing anchorage independence, albeit with different efficiencies. Colonies produced by the MC29- and MH2-infected NR cells were larger and more numerous than those produced by PR-A-infected cells. Qualitatively similar results were obtained in independent experiments carried out with quail embryo NR cells (data not shown). To analyse the relationship between induced growth transformation and levels of expression of oncogene products, MC29- and MH2-infected cells were labelled with ^{35}S-methionine and immunoprecipitated with the appropriate antibodies. Infected cells expressed levels of the putative tranforming products[5]

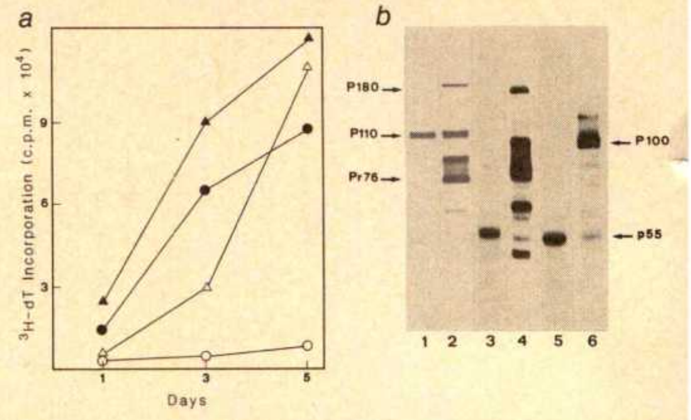

Fig. 1 *a*, Kinetics of DNA synthesis in NR cells infected with viruses. NR cells were infected with RAV-1 (○), PR-A (●), MC29 (△) and MH2 (▲), then passaged twice, seeded at 5×10^4 per well on collagenated clusters and finally labelled with ^{3}H-dT as indicated below. *b*, Analysis of viral proteins in NR cells (5th passage) infected with MC29 (lanes 1, 2), MH2 (lanes 3, 4) and non-producer chick embryo fibroblasts transformed by MH2 (lanes 5, 6).

Methods. For transformation of NR cells and ^{3}H-thymidine incorporation, neural retinae (NR) were dissected from 7-day-old chicken or quail (*Coturnix japonica*) embryos and cell suspensions prepared by using standard procedures. After washing, the cells were suspended in Dulbecco's modified Eagle's medium (DMEM, Flow) containing 0.2% fetal calf serum (FCS, Flow) and plated 5×10^6 cells per 60-mm collagen-coated (Vitrogen, Flow) tissue culture dish (Falcon) to promote rapid attachment to the substrate. The cells were thereafter cultured in growth medium (DMEM containing 10% FCS, 1% chicken serum, 10% triptose phosphate broth) on collagen-coated dishes and fed every other day. Primary cultures were infected at high multiplicity of injection (0.1–1.0) with the following viruses: RAV-1, PR-A, MC29(RAV-1) and MH2(RAV-1). The origin of the viral stocks is described in refs 23 and 24. Control and virus-infected cultures were maintained at 37 °C. Third-passage cultures were labelled for 2 h, at day 1, 3 and 5 after seeding, with ^{3}H-thymidine (NEN, 77 Ci mmol^{-1}), using 2 μCi ml^{-1} in growth medium supplemented with 2 μM cold thymidine. Following incubation, cultures were washed successively in cold phosphate-buffered saline (PBS), 5% trichloroacetic acid (TCA) and methanol; cells were then solubilized in 0.1 M NaOH and counted in Picofluor (Packard). For cell labelling and immunoprecipitation, fifth-passage, infected NR cells were washed twice with methionine-free MEM and then exposed to the same medium also containing 100–200 μCi ml^{-1} of ^{35}S-methionine (NEN). Incubation was for 1 h at 37 °C. After labelling, cultures were washed twice with PBS and frozen *in situ* at −80 °C until used for further analysis. Labelled cells for immunoprecipitation were collected and lysed in RIPA buffer[31], also containing 0.2% Trasylol, 0.2mM phenylmethylsulphonyl fluoride (PMSF) and 5 mM EDTA. After clarification at 13,000 r.p.m. for 30 min in a microcentrifuge, aliquots of extracts, normalized for TCA-precipitable counts, were challenged with 3–5 μl ml^{-1} of either a serum raised in rabbits against a bacterially expressed viral *myc* protein (obtained from K. Moelling) (lanes 1, 3, 5) or an anti-*gag* serum (lanes 2, 4, 6) and the mixtures incubated on ice for 1 h. Immunoprecipitates were collected with Pansorbin (Calbiochem), washed and dissolved by boiling in SDS-sample buffer. Proteins were resolved by electrophoresis on 8% SDS-polyacrylamide gels[32] followed by fluorography and autoradiography. Immunoprecipitates analysed in lanes 1 and 2 were run on a separate gel.

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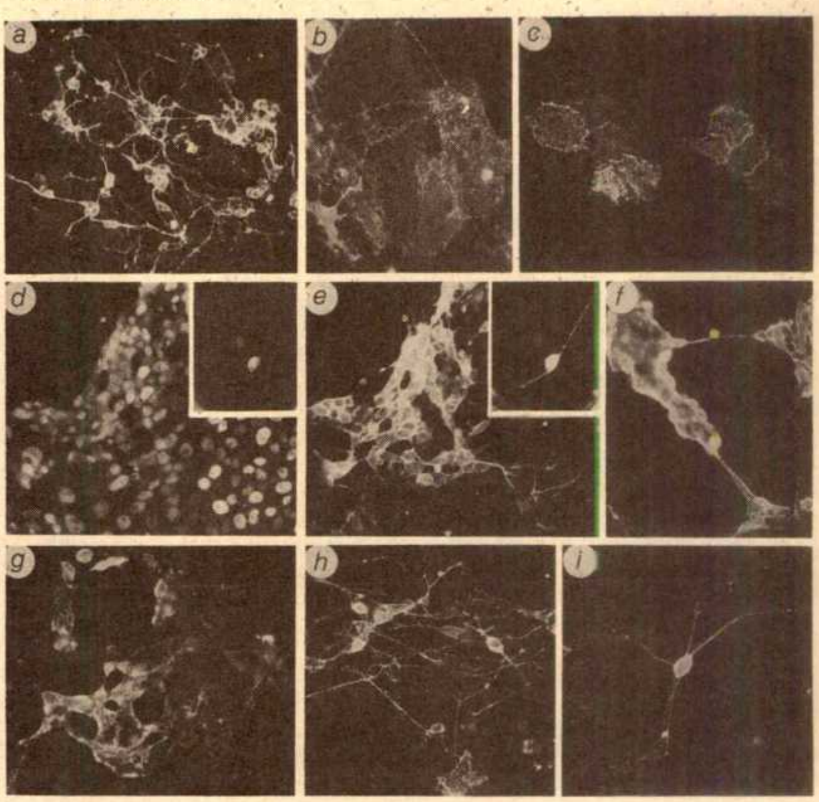

Fig. 2 Indirect immunofluorescent visualization of neural specific antigens in uninfected and virus-infected NR cells. *a–c*, Primary (*a*, *b*) or fifth passage (*c*) control retinal cells were stained with the monoclonal antibodies A2B5 (*a*, *b*) or Leu7 (*c*). *d*, *e*, Same field of 7th passage MC29-infected NR cells double-stained with both anti-v-*myc* serum (*d*) and monoclonal antibody A2B5 (*e*). The enlarged insets clearly show a cell with a neuronal phenotype stained by both antibodies. *f–i*, NR cells infected with (*f*, *g*) MC29 or (*h*, *i*) MH2 and stained with A2B5 (*f*, *h*) or Leu7 (*g*, *i*). **Methods.** Uninfected or virus-transformed cells cultured on collagen-coated 35-mm dishes were labelled with A2B5 ascite fluid (1:50) (prepared from cells obtained from the American Type Culture Collection) or Leu7 (1:5) (from A. Mudge) and, after fixation for 10 min with 3.3% paraformaldehyde in PBS, stained with goat anti-mouse immunoglobulin antibodies conjugated to fluorescein (Miles, 1:30). For double-staining with A2B5 and anti-*myc* (1:50) the same slide was fixed as above, and permeabilized with 0.5% Triton X-100 (BioRad) in Tris-buffered PBS. Second antibodies were affinity-purified goat anti-rabbit antibodies conjugated to rhodamine (Zymed, 1:50) and goat anti-mouse immunoglobulin conjugated to fluorescein. After washing, cultures were mounted in Gelvatol and examined in a Leitz Dialux fluorescence microscope.

$P110^{gag\text{-}myc}$, $p55^{myc}$ and $P100^{gag\text{-}mil}$ which were comparable to those of infected fibroblasts (Fig. 1*b*). In addition we attempted to localize v-*myc* protein in infected cells by immunofluorescence of fixed cells. Antisera against bacterially expressed v-*myc* protein stained nuclei[6] strongly in all MC29- (Fig. 2*d*) and MH2-infected (data not shown) NR cells.

We next attempted to characterize the lineages of NR-transformed cells. The neuroretina of the 6–7th-day avian embryo consists of cells with different capacities for self-renewal and differentiation[7,8]. Progenitor cells that can be grown *in vitro* include presumptive glia cells of the Muller type[9] and cells which, when seeded on the proper substrate, give rise to cells with a neuronal phenotype as defined by a round, refractile body with thin neurites[10]. NR cells infected by PR-A gave rise to a uniform population of moderately transformed cells capable of reverting to a flat, glial-like phentoype when shifted to the non-permissive temperature in *ts*-RSV-infected cells (data not shown and ref. 11). MC29- and MH2-infected cells were small and had prominent nucleoli, a hallmark of v-*myc*-transformed cells[12]. Moreover, cultures infected with the latter viruses consisted of a carpet of epitheliod, flat cells underlying a population of process-bearing cells, with a contained body. MC29- and MH2-transformed NR cells continued to express the high-affinity, Na-dependent D-aspartate (a non-metabolized analogue of the neurotransmitter L-glutamate) and γ-aminobutyric acid (GABA) transport systems that have been described for NR cells in culture[13,14], whereas the same activities were largely inhibited by v-*src* (Table 1). Autoradiographic experiments showed that uptake of D-aspartate was a property of flat glia-like cells, but GABA uptake, as in primary cultures, was confined to a subset of neuron-like cells which had grown long processes (not shown).

Table 1 Agar colony formation and uptake of neurotransmitters by control and virus-infected NR cells

Virus	Agar colonies % efficiency	D-Asp uptake	GABA uptake
Uninfected	0	10.5	1.4†
RAV-1	0	8.6	n.d.
PR-A	10^{-4}*	0.9	0.5
MC29	2×10^{-3}	25.8	9.2
MH2	10^{-2}	16.2	4.1

* Large (> 0.25 mm) colonies only.

† Low levels of GABA uptake in control cells are due to loss of neurons on passaging.

Uptake of D-Asp and GABA is expressed in nmol min^{-1} per mg protein; n.d., not determined. Agar colony assays were performed on fifth-passage control and infected NR cells as described[24]. Plates were scored for colonies 14 days after seeding. For the uptake determination and autoradiography, fifth-passage cells were rinsed twice with a HEPES-buffered medium (137 mM NaCl, 2.7 mM KCl, 1.14 mM KH_2PO_4, 6.45 mM Na_2HPO_4, 0.5 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose) and then incubated for up to 30 min at 37 °C in ^3H-D-aspartic acid (2 μCi ml^{-1}; specific activity 20 Ci $mmol^{-1}$, NEN) or ^3H-GABA (2 μCi ml^{-1}; specific activity 33 μCi $mmol^{-1}$, NEN). After incubation the dishes were washed three times with warm medium and either fixed for autoradiography or solubilized in 0.1 M NaOH and counted in Picoflour (Packard). The dishes were processed for autoradiography as in ref. 30.

Uninfected and virus-infected cells were also characterized for the expression of tissue-specific antigens. In Fig. 2*a*, a cluster of NR neurons from a primary culture, recognizable by their round cell body and extensive process formation, are shown on a background of flat non-neuronal cells. In *a* only the neurons were stained by monoclonal antibody A2B5, which recognizes a tetrasialoganglioside unique to neural cells[15], and Leu7 which labels neuroectodermal cells[16] (not shown). In primary cultures only a proportion of flat glia cells were stained by A2B5 (Fig. 2*b*) or Leu7 and this property was progressively lost *in vitro* so that <5% were positive at later passages (Fig. 2*c*). Double immunofluorescence studies (Fig. 2*d*, *e*) showed the simultaneous presence of $P110^{gag\text{-}myc}$ protein and A2B5 antigen in a large proportion of flat and process-bearing MC29-transformed cells; as in uninfected primary cultures, a restricted fraction of neuron-like cells expressed the 70K subunit of neurofilaments (data not shown). Expression of these markers although maintained in process-bearing MC29- (Fig. 2*f*, *g*) and MH2-

transformed cells (Fig. 2*h*, *i*), was not detectable in PR-A-transformed cells (data not shown and ref. 17).

The present data provide strong evidence that in primary chick NR cells the v-*myc* oncogene does not require the action of a complementary oncogene such as v-*mil* to induce two major traits associated with loss of growth control in transformed cells. Although entirely consistent with the conclusion that the v-*mil* oncogene of MH2 appears non-essential for transformation of fibroblasts[18] and macrophages[19] *in vitro*, our results differ considerably from the experiments of Bechade *et al.*[2], where infection of chick NR cells with MC29 and deletion mutants of MH2 expressing only the v-*myc* oncogene had no apparent effect on these cells. Two points relevant to this issue may be made. The first suggests that part of the apparent discrepancy may have arisen from our improved culture conditions in which efficient virus spread allows a rapid and uniform viral infection. A second, complementary argument stems from our observation that in chick NR cells mitogenic stimulation by v-*myc*, in contrast to v-*src* or other oncogenes, becomes apparent only after expression of the transforming protein by the majority of the cells (Fig. 1*a* and data not shown). This behaviour is not observed if NR cells are infected with a retrovirus such as MH2 expressing both the v-*myc* and the v-*mil* oncogenes (this study and ref. 2). At a superficial level, this property suggests the existence of environmental cues that delay the expression of the transformed phenotype in MC29-infected NR cells.

That primary avian cells belonging to different lineages are, with no known exception, efficiently transformed *in vitro* by retroviruses carrying the v-*myc* oncogene alone[20–24] contrasts with the observation that in non-established rodent fibroblasts transfected v-*myc* usually requires a cotransforming oncogene of the *ras* type[25,26]. In one instance, a v-*myc*-containing recombinant retrovirus was able to confer anchorage-independence on non-established mouse fibroblasts and macrophages[27]. A possible resolution for the incompletely transformed phenotype of v-*myc*-bearing mammalian cells may come by considering that (1) mammalian cells express comparatively lower levels of exogenous v-*myc*[27] and (2) normal adjacent cells appear to exert a 'suppressive' effect on the focal outgrowth of mammalian cells bearing v-*myc*[28]. Accordingly, rare full transformation of rat embryo fibroblasts seems to require both elimination of adjacent cells and high levels of expression of the v-*myc* protein[28].

Interestingly, at the biochemical level, expression of v-*myc* did not appreciably reduce the high-affinity uptake of D-aspartate or GABA, even though the same activities were severely affected by v-*src*. Furthermore, the expression of markers of neurons and glia cells was not appreciably affected, except for changes that could be attributed to the high proliferation rate of transformed cells. Indeed, the specific activities of neurotransmitter uptake and the immunofluorescence studies suggest that, on passaging, the *myc*-transformed cells retained their normal differentiated phenotype better than the uninfected cells. This is not surprising in view of previous work indicating that, in contrast to v-*src*, v-*myc* does not depend on blocking differentiation to promote transformation[21–24,29].

Regarding the origin of the cells acting as targets of MC29 and MH2 infection, at present we feel that the evidence is not sufficient to discriminate between infection of separate precursors or of a common progenitor to both glia cells and neurons. In any event, the feasibility of conferring proliferative potential on neuroectodermal cells without grossly interfering with their differentiated phenotype, suggests a novel experimental approach to generating clonal strains from short-lived, early neural precursor cells otherwise not easily amenable to adequate characterization.

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Retroviral transduction of T-cell antigen receptor β-chain and *myc* genes

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Support for multistage models of oncogenesis has been provided by several highly leukaemogenic retrovirus isolates that have transduced more than one host cell gene[1–5]. Where functional studies have been performed, these retroviral oncogenes show synergy for *in vitro* transformation and leukaemogenesis[6,7]. In naturally occurring feline leukaemias associated with feline leukaemia virus (FeLV), retroviral transduction of *myc* is a frequent oncogenic mechanism[8–10]. But evidence suggesting that the FeLV v-*myc* genes might be insufficient for leukaemogenesis was provided by the latency (12 weeks) and clonality of FeLV/v-*myc*-induced tumours and the absence of demonstrable *in vitro* transformation by these viruses[11]. In the search for secondary leukaemogenic events in FeLV/v-*myc* tumours, we have identified a case of FeLV transduction of a T-cell antigen receptor β-chain gene. The proviruses carrying this gene (which we have named v-*tcr*) were a separate population from those carrying v-*myc*. In its normal role, the T-cell receptor β-chain forms part of a multimeric complex involved in antigen recognition[12–14] and T-cell activation[15,16]. We suggest that v-*tcr* is a novel viral oncogene which assisted v-*myc* in the genesis of a naturally occurring case of thymic lymphosarcoma.

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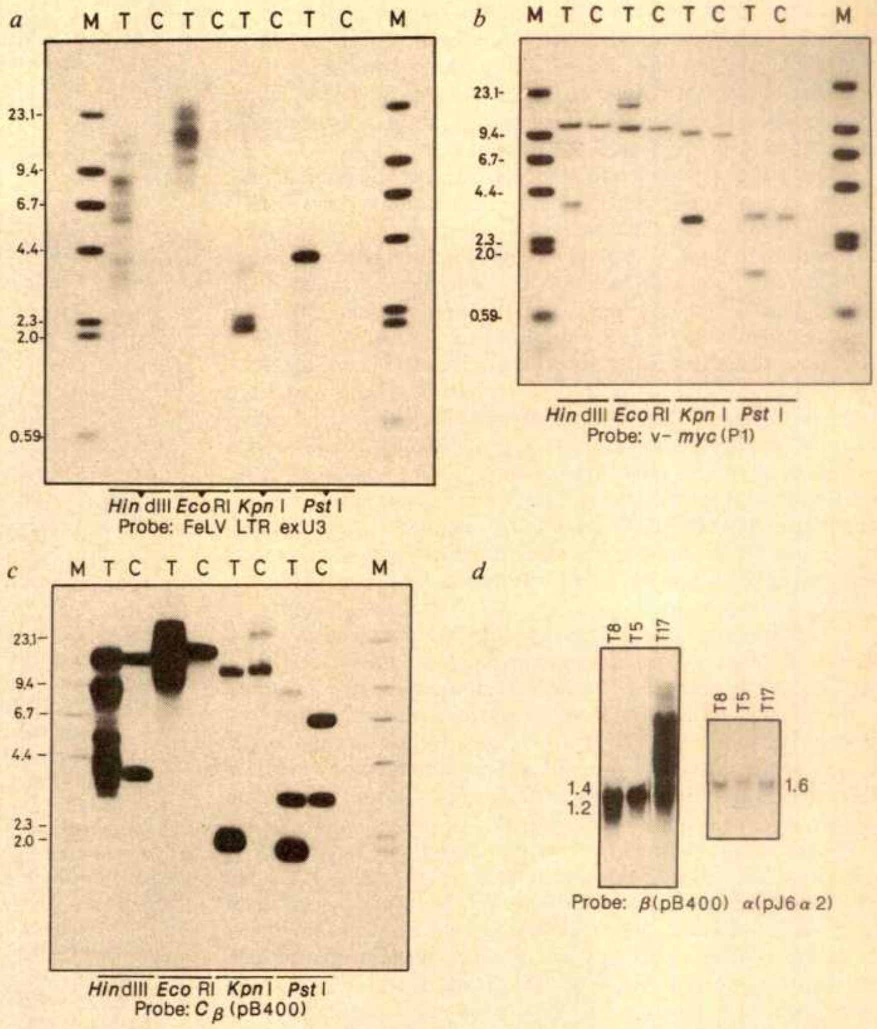

Fig. 1 Evidence for FeLV proviruses carrying *myc* and T-cell antigen receptor β-chain genes in feline thymic lymphosarcoma T17. Restriction enzymes such as *Kpn*I and *Pst*I have conserved sites in FeLV long terminal repeats[42] and generate common internal proviral fragments regardless of integration site. On digestion, multiple proviral copies yield a single strongly hybridizing restriction fragment. In contrast, *Eco*RI is generally a non-cutter in FeLV so that the fragment detected by Southern blot hybridization is different for each integrated copy but of greater length than the FeLV provirus. Hybridization was carried out with: *a*, an FeLV LTR probe from the U3/R region[43]; *b*, a probe derived from exon 2 and 3 domains of FeLV CT4 v-*myc*[8,20]; and *c*, a constant region fragment from a cDNA clone of human T-cell antigen receptor β-chain gene (pB400)[19]. In *a–c*, T denotes tumour DNA and C, control tissue (kidney) DNA; M, size markers (end-labelled λ *Hin*dIII fragments). *d*, Northern blot analysis of feline T-cell tumour RNAs for α- and β-gene transcripts. RNAs from thymic lymphosarcomas T5 and T8 are shown alongside T17 to exemplify the typical transcript patterns we found in a survey of ~30 tumours (ref. 11 and unpublished results).
Methods. For Southern blots in *a*, *b* and *c*, 20 μg DNA for each lane was digested with the appropriate restriction enzyme. Samples were separated on 0.8% agarose gels and transferred to GeneScreen membranes (NEN). Hybridization was performed overnight at 42 °C in a solution containing 5 × SSC, 5 × Denhardt's solution, 0.1 M sodium phosphate, 0.1% SDS, 10% dextran sulphate and 100 μg ml^{-1} denatured salmon sperm DNA. The blots were washed at 60 °C for 1 h in 0.5% SDS and (*a*) 0.2 × SSC, (*b*) 2 × SSC and (*c*) 0.1 × SSC, before final rinsing, drying and exposure to Kodak XAR-5 film at −70 °C. For the Northern blot analysis in *d*, 20 μg samples of total cellular RNA were electrophoresed on 1% agarose gels containing 2.2 M formaldehyde, transferred onto GeneScreen membranes and hybridized with α- and β-chain gene probes essentially as for Southern blots. Filters were washed in 2 × SSC, 0.5% SDS at 60 °C (3 × 25 min). The transcript sizes were estimated relative to radiolabelled *Hin*dIII-digested λ DNA markers (not shown). Membranes were exposed to Kodak XAR film at −70 °C for 24 h (T_β) or 48 h (T_α).

Tumour T17 was a field case of thymic lymphosarcoma in a young adult male cat. The case was unusual in that the cat had no demonstrable FeLV viraemia or viral antigen in blood plasma, although high-titre FeLV-neutralizing antibodies were present. The tumour cells harboured multiple FeLV proviruses which were highly expressed as intracellular RNA and viral protein (Fig. 1 and unpublished results). The tumour cells were readily established in culture with no requirement for exogenous interleukin-2 (IL-2), but released only very low titres of infectious FeLV (less than 10 focus-forming units (f.f.u.) per ml). This pattern of *in vitro* growth without IL-2 was characteristic of other T-cell tumours induced by FeLV/v-*myc* viruses[11], and we found that T17 tumour DNA also harboured FeLV recombinant proviruses carrying the *myc* gene (Fig. 1*b*).

Our evidence for derangement of a T-cell antigen receptor (*Ti*) gene in tumour T17 came from a larger study in which we assessed the phenotype and maturity of a series of feline T-cell tumours by hybridization analysis of the rearranging genes (α-chain and β-chain) of the T-cell receptor complex. The α- and β-chain genes are sequentially rearranged and expressed during thymocyte ontogeny (β then α)[17-19]. These studies were facilitated by the finding that the feline *Ti* genes can be detected readily using probes derived from the human *Ti* genes and that the feline genes have similar structural organization and transcript sizes to the human genes. Most of the feline thymic tumours examined, notably those involving altered *myc* genes (c-*myc* or v-*myc*) expressed both α- and β-gene transcripts of the expected size and demonstrated clonal patterns of β-gene rearrangement. Tumour T17, however, showed around tenfold amplification of β-chain DNA sequences; further restriction mapping provided preliminary evidence that FeLV proviral capture was the basis for this amplification (Fig. 1*c*). Also, the β-gene transcripts were much larger and more abundant than normal (Fig. 1*d*), again consistent with their expression as part of an FeLV provirus. In contrast to β, the expression of the α-gene in T17 appeared to be normal (Fig. 1*d*).

For more detailed analysis, we generated a bacteriophage library from T17 DNA and screened with probes including C_β (human β-chain constant region)[19], v-*myc*[8,20] and an exogenous FeLV-specific *env* gene probe[21]. By this approach we have cloned FeLV proviruses carrying the β-chain sequences, proviruses carrying v-*myc* and other proviruses that appear to be typical helper-type FeLV. None of the clones hybridized to both *myc* and C_β, confirming the prediction from Southern blot analysis that the two host genes were on separate proviral elements (Fig. 1).

The clones T17T-22 and T17T-31 contained indistinguishable defective FeLV proviral structures incorporating the C_β-hybridizing sequence at the 3′ end in place of *env* sequences (Fig. 2). Their internal structures were as expected from Southern blot analysis of tumour DNA (Fig. 1*c*). A 1.9-kilobase (kb) *Pst*I fragment of T17T-22 was subcloned into plasmid pUC18 for DNA sequence analysis. The sequence of this fragment (Fig. 3) shows a rearranged, joined and spliced version of a β-chain gene in place of the 3′ end of the *pol* and most of the *env* gene of FeLV. The v-*tcr* sequence lacks the intron sequences characteristic of known β-chain gene loci, presumably as a result of replication through an RNA intermediate.

The 5′ FeLV-host junction point is within *pol* and is coincident with that recorded in the FeLV/*myc* recombinant virus, FTT[22] (Fig. 3*b*) and within 16 base pairs (bp) of another FeLV/*myc* 5′ junction, in the CT4 isolate[20]. This identifies a second virus-host junction 'hot-spot' in FeLV, the other being located in the $p30^{gag}$ gene[23]. This may represent a favoured recombination site or alternatively may reflect a requirement for expression of the transduced host gene from the presumptive *env* messenger RNA splice acceptor site (at position −15 relative to the illustrated sequence). The acquired host sequence includes an open reading frame of 963 bp that encodes a full-length β-chain gene product. Sequences 3′ to the v-*tcr* open reading frame show weak interspersed homology to murine C_β 3′ untranslated sequences[24,25]. A better match can be obtained to $C_{\beta 2}$ than to $C_{\beta 1}$ but because there is such divergence between all three sequences, direct analysis of the feline C_β genes will be required to establish the precise genetic origin of v-*tcr*.

A full-length β-chain gene product of 331 amino acids would be encoded by a spliced, subgenomic mRNA if the nearby FeLV splice acceptor site is used. Otherwise, the presumed *pol* reading frame (identified by homology to murine leukaemia virus[20,26]) is coincident with that of the v-*tcr* gene. Thus, a *gag-pol-tcr* fusion product may be generated. This depends, however, on the integrity of the *pol* reading frame upstream of the region sequenced.

Within the predicted FeLV v-*tcr* product, we see close homology to human and murine β-gene products. The open reading frame encodes a hydrophobic N terminus or leader sequence, which may be expected to mediate membrane insertion, followed by a sequence which shows clear structural hallmarks of a V_β gene product. This is shown in Fig. 4 as an alignment with a murine V_β gene product. A strikingly close alignment is possible with a murine V_β sequence obtained from BALB/c thymocytes (TB21)[27]. The v-*tcr* *V* domain falls into the class I V_β genes defined by Schiffer and colleagues[28], and has all the expected conserved sequence hallmarks presumed to be important for V_β secondary structure and the generation of the antigen binding site.

Much of the diversity of β-chain sequences that should account for immunological specificity appears to be generated by *V-D-J* joining[27-30]. Sequences that appear to be derived from *D* and *J* elements are also seen in FeLV v-*tcr*. The derived amino-acid sequence from the presumptive J_β segment also shows good alignment with human and mouse sequences. The best match is to the murine $J_{\beta-1(86T1)}$ sequence[24,31] (Fig. 4). The D_β coding sequences are intrinsically more variable, presumably because of the small size of the core elements and the possibility of nucleotide additions during gene rearrangement[27-30]. However, because the v-*tcr* V_β and J_β homologous regions are interrupted, we expect that the interposed sequence was derived from a D_β element.

The C-terminal portion of the predicted v-*tcr* product shows even closer homology to human and murine C_β products[24,25,32,33] (Fig. 4). The differences between the v-*tcr* and human C_β sequences are slightly fewer than those between human and mouse C_β and no more than would be expected between the germline genes of these different animal species. Again, the important sequence hallmarks such as the cysteine residues are conserved, as is the putative C_β transmembrane domain at the C terminus.

There is a difference in the transmembrane domain which may be important when considering the possible transforming potential of the v-*tcr* gene. This is a non-conservative change in v-*tcr* relative to the human and mouse coding sequences (Met → Lys 325). Although this substitution does not alter the predicted α-helical structure, the charge alteration results in a significantly altered hydrophilicity profile. We draw comparison with the *neu* oncogene, a homologue of the epidermal growth factor receptor, where activation for *in vitro* transformation has been reported to result from a point mutation (Val → Glu) in the transmembrane coding region[34]. In the human T-cell receptor (CD3(T3)-Ti) complex, there is normally a balance of charges within the transmembrane domain (three positive, three negative[35-39]): adding an additional positive charge (Lys) could conceivably alter intermolecular interactions in the complex.

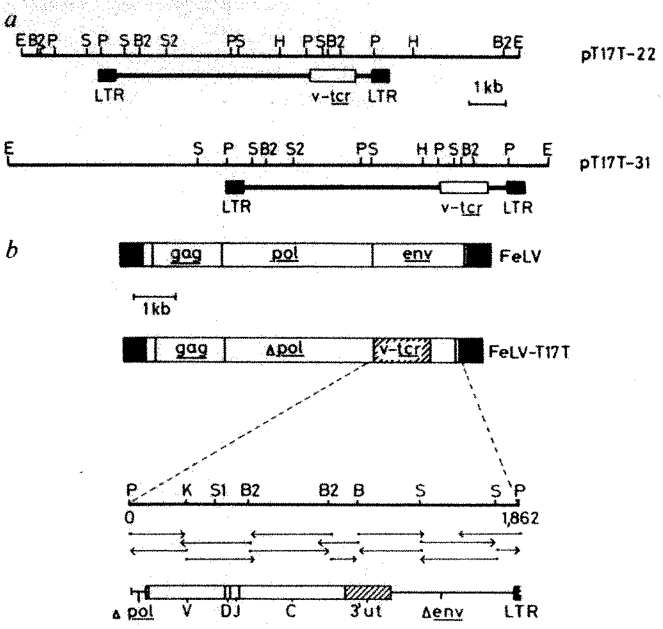

Fig. 2 *a*, Restriction maps of selected molecular clones. Line below map, position of FeLV genome with (black boxes) LTR regions and (open box) v-*tcr* gene. Restriction enzyme sites shown: B2, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; S1, *Sst*I; S2, *Sst*II and P, *Pst*I. *b*, Genetic location of the β-chain gene sequences within FeLV T17T. A typical replication-competent FeLV (dimensions based on FeLV-A/Glasgow-1[21]) is shown above for comparison. The 1.9-kb *Pst*I fragment is also shown in larger scale below, with (arrows) sequencing strategy and the coding potential of the host-drived sequence indicated by boxes. Restriction enzyme abbreviations as in *a*, except B, *Bam*HI; K, *Kpn*I.

Methods. To clone the novel proviral elements from T17 DNA, a bacteriophage library was constructed from DNA of tumour T17 using the *Eco*RI cloning vector λEMBL4 (Northumbria Biologicals). Tumour DNA was digested to completion with *Eco*RI, and size-selected (10–20 kb) on low-melting-point agarose gels before ligation to phage arms. Around 1×10^6 recombinants were obtained from 2 μg of size-selected DNA. Several probes were used to screen the library, including the C_β probe, an FeLV v-*myc* probe, an FeLV LTR (U3) probe, and an exogenous FeLV-specific *env* gene probe[21]. Cross-hybridization analysis showed that most of the clones selected with *myc* and C_β probes also hybridized to FeLV LTR but not to *env* gene probes. The *Eco*RI bacteriophage vector inserts were subclones into pUC18 and mapped by double digest and partial digest analysis. The location and orientation of FeLV and β-chain gene sequences were deduced from hybridization analysis of the cloned DNAs and the presence of characteristic restriction enzyme cleavage sites.

The occurrence of a transduced host gene within a retrovirus in a naturally occurring tumour does not in itself constitute proof of a direct oncogenic function, although precedent strongly suggests that this is the case. When considering possible functions of the FeLV v-*tcr* gene product in oncogenesis, we must consider the properties of the T-cell antigen receptor complex. In this multimeric structure, two predominantly extracellular chains (α and β) are required for antigen recognition[12-14] and several other transmembrane proteins (γ, *a*

```
CTGCAGCTGGGTCCAAGAAGAGATCCAGAGACCTCTAGCGGCGGCCTACCGAGAAAAGCT    60

CGAAACCCCGGTTGTGCCCCACCCCTGAGCTGGATGATATCTTGGCTTCCCAGTGTTGCC   120
 FeLV pol              -> |       MetIleSerTrpLeuProSerValAla     9

ATGGGCTCCAGGCTTCTCTGCTGCGTGGCCCTTTGTCTTCTGGGAGCAGGCCCAGCGGAT   180
MetGlySerArgLeuLeuCysCysValAlaLeuCysLeuLeuGlyAlaGlyProAlaAsp    29
                              LEADER                ->

TCCGGACTCACCCAGACTCCAAGACACCTGGTCAAGGCAAGAGGACAGCAAGTGACGCTG   240
SerGlyLeuThrGlnThrProArgHisLeuValLysAlaArgGlyGlnGlnValThrLeu    49
               <--    V-REGION    -->

AGTTGTTTCCCTATCTCTGGACACCTGTCTTTGTACTGGTACCAACAGGCCGTGGGCCAG   300
SerCysPheProIleSerGlyHisLeuSerLeuTyrTrpTyrGlnGlnAlaValGlyGln    69

GGCCCCCAGTTACTCATTCAGTATTACAACAGGGAAGAGAGAGGCAAAGGAAACTTCCCG   360
GlyProGlnLeuLeuIleGlnTyrTyrAsnArgGluGluArgGlyLysGlyAsnPhePro    89

GAACGATTCTCGGCTCAACAGTTCCCTGACTCTCACTCTGAGCTAAACATGACCTCCTTG   420
GluArgPheSerAlaGlnGlnPheProAspSerHisSerGluLeuAsnMetThrSerLeu   109

GAGCTCACGGATTCGGCCCTCTATCTCTGTGCCAGCAGCCCTAATGAGGACAGCGAATAT   480
GluLeuThrAspSerAlaLeuTyrLeuCysAlaSerSerProAsnGluAspSerGluTyr   129
                                    |<-- D         ->|<-

GGCGAGACCCTCTATTTTGGGGAAGGGAGCCGGCTCACTGTTGTAGAGGACCTGAAAAAG   540
GlyGluThrLeuTyrPheGlyGluGlySerArgLeuThrValValGluAspLeuLysLys   149
              J                        -->|<-- C-REGION

GTGAGCCCTCCCAAGGTCACAGTGTTGCAACCATCAGAAGCAGAGATCTCCCGGACCCTG   600
ValSerProProLysValThrValLeuGlnProSerGluAlaGluIleSerArgThrLeu   169

AAGGCCACGCTCGTGTGCCTGGCCACAGGCTTCTACCCCGACCACGTGGAGCTGAGCTGG   660
LysAlaThrLeuValCysLeuAlaThrGlyPheTyrProAspHisValGluLeuSerTrp   189

TGGGTGAACGGGAAGGAGGTGCGCGACGGGGTCAGCACGGACCCTGAGCCCTACAAGGAA   720
TrpValAsnGlyLysGluValArgAspGlyValSerThrAspProGluProTyrLysGlu   209

CAGTCCGGCGCGAATGTCTCCAGCTACTGTCTGAGCAGCCGGCTGAGGGTCTCCGCCACC   780
GlnSerGlyAlaAsnValSerSerTyrCysLeuSerSerArgLeuArgValSerAlaThr   229

TTCTGGCACAACCCTCGGAACCACTTCCGCTGCCAGGTCCAGTTCCACGGGCTCGGGAAG   840
PheTrpHisAsnProArgAsnHisPheArgCysGlnValGlnPheHisGlyLeuGlyLys   249

GATGACCAGTGGGACTACCCCGAGGCCAAACCCGTCACCCAGAACGTCAGTGCTGACACC   900
AspAspGlnTrpAspTyrProGluAlaLysProValThrGlnAsnValSerAlaAspThr   269

TGGGGCAGAGCAGACTGTGGCTTCACTTCAGCGTCCTACCAGCAGGGGGTCCTGTCGGCC   960
TrpGlyArgAlaAspCysGlyPheThrSerAlaSerTyrGlnGlnGlyValLeuSerAla   289

ACCATCCTCTATGAGATCTTGCTGGGGAAGGCCACCCTATATGCCATCCTGGTCAGTGTC  1020
ThrIleLeuTyrGluIleLeuLeuGlyLysAlaThrLeuTyrAlaIleLeuValSerVal   309

CTCGCGCTGATGGCCAAGGTCAAGAGAAAGGATTCCTGAAGCCAGCTCGGAGGAGGAGTC  1080
LeuAlaLeuMetAlaLysValLysArgLysAspSer      3'UT                 331

CTGAGCTTCCAGTCGGCCATTGCCCCAATGTGGATCCTCCTTTTCCCACTTAGAGCCAGT  1140

GCTTCTGAAGAGCTGTTTTCATCCCTCTGCTCACCCCGGTAGCTACCCCTTATGTGCATG  1200

CACACGCGGTCGCTCACGACTCAACTTGCCCTAAGCCAGGGGGACCTCGCGTGACGAAAT  1260

TGACTGAATCAATAAAAATGGCCATGCACACAGACATCCAGGCCCTAGAGGAGTCAATTA  1320
            -->¦<-FeLV del p15E                        A  A

GTGCCTTAGAAAAGTCCCTGACCTCCCTTTCTGAAATAGTCTTACAAAACAGACGGGGCC  1380
                                  G

TAGATATTCTATTCTTACAAGAGGGAGGGCTCTGTGCCGCATTAAAAAAGGAATGTTGCT  1440
                                             G   G A

TCTATGCGGATCACACCGGACTCGTCCGAGACAATATGGCTAAGTTAAGAGAAAGACTAA  1500
                                         C

AACAGCGGCAACAACTGTTTGACTCCCAACGGGGATGGTTTAAAGGATGGTTCAACAAGT  1560
                               A          G

CCCCCTGGTTTACAACCCTAATTTCCTCCATTATGGGCCCCTTACTAATCCTACTCCTAA  1620

TTCTCCTCTTCGGCCCATGCATCCTTAACCGATTAGTACAATTCGTAAAAGACAGAATAT  1680

GCAAATCCCCCCGACAGAATATCTGTGGTACAAGCCTTAATTTTAACCCAACAGTACCAA  1740
 ************                         G  T

CAGATAAAACAATACGATCCGGACCAACCATGATTTCCAATTAAATGTAAGATTCCATTT  1800
     G                  G                          T

AGTCCCCAGAAGAAGGGGGGAATGAAAGACCCCCCACCCCAAAATCTAGCTAGCTACTGCAG 1862
          A           |LTR      T           T         C
```

b

```
5' Junction

FeLV   AAGCTCGAAACCCCGGTCGTGCCTCACCCCTTCAAACCAG
CT8

CT4    AAGCTCGAACCCC                              - FeLV
           gaagggagaTCCGGAGTGACCG                 - v-myc

T17T   AAGCTCGAAACCCCGGTTGTGCCCCACCCCT            - FeLV
                                      GAGCTGGAT   - v-tcr

FTT    AAACTTGAAACCCCGGTTGTGCCTCACCCCT            - FeLV
                              cggacgctGGATTTCCT   - v-myc


3' Junction

Cβ2      GAAACAGACTAAATCAATAAAAACATGGAGTTAACCTGGT
         ||||  |||| |||||||||||||     |   | | 
v-tcr    GAAATTGACTGAATCAATAAAAATGGCCATGCACACAGAC
               |     |  | ||  |||||||||||||||||||||
FeLV     AGCCCAGTTCAGACAACTACAAATGGCCATGCACACAGAC
(G1)
```

Fig. 3 *a*, Sequence of the T-cell antigen receptor β-chain gene and surrounding FeLV sequences in FeLV T17T. Presumptive limits of leader, variable (*V*), diversity (*D*), joining (*J*) and constant (*C*) regions of the FeLV v-*tcr* product are shown below the sequence. In the p15E coding region 3′ to the v-*tcr* sequence, base mismatches relative to FeLV-A/Glasgow-1 are indicated under the sequence. Asterisks, a short insert relative to FeLV-A/Glasgow-1[21]; boxes, duplication of the surrounding p15E coding sequence. These changes disrupt the p15E reading frame. *b*, Virus-host junctions of FeLV-T17T with comparison to other host-gene-transducing FeLV isolates. Above, the 5′ junction points are shown for FeLV-T17T and for two v-*myc*-containing FeLV isolates, CT4[20] and FTT[22]. The sequence of the 3′ *pol* region of an FeLV isolate (CT8)[8,20] is shown above for comparison. The junction point is identical for T17T and FTT, and within 18 bp of the CT4 junction point. Also included are the host DNA sequences immediately 5′ to the recombination site (lower case letters) where these have been determined. Below, the 3′ junction point is illustrated, with the v-*tcr*, a homologous region at the 3′ end of the murine $C_{\beta 2}$ gene[24] shown (above) and the FeLV-A/Glasgow-1 p15E coding sequence[21] shown (below) for comparison. Box, an 8-bp stretch in which FeLV and $C_{\beta 2}$ match at six out of eight bases. The feline C_{β} sequences have not yet been analysed.

Methods. For sequencing, a 1.9-kb *Pst*I fragment of FeLV T17T-22 (Fig. 3) was subcloned into plasmid pUC18, before further subcloning into M13 mp18 and mp19[44] and sequencing by the dideoxy method[45]. Sequence was determined unambiguously on both strands using the cloning sites indicated. An open reading frame for a potential β-chain T-cell antigen receptor gene product of 331 amino acids spans nucleotides 93–1056.

δ and ε) appear to be induce in transducing growth signals into the cell[15,16]. On the published evidence, it is difficult to conceive of a free β-chain gene product providing a growth stimulus in the absence of other components of the complex. Consequently, the transforming potential of FeLV T17T may prove to be limited to mature T cells. In accord with this suggestion, the T17 tumour cells appear relatively mature by the criterion of α-chain gene expression (Fig. 1*d*).

How might the FeLV v-*tcr* gene product disrupt the normal functioning of the receptor complex? We favour a model in which the FeLV v-*tcr* product is expressed as a component of the receptor complex which it activates in the absence of antigen binding. However, it is possible that the FeLV v-*tcr* product may be altered in structural location, conformation and/or transport to the cell surface. All of these properties may be changed if the gene product is expressed as a *gag–pol–tcr* fusion protein. From studies on the requirements for *in vitro* proliferation of T lymphocytes it seems that triggering through the antigen receptor complex can induce an IL-2-dependent autocrine pathway[40]. Also, v-*myc* genes release murine cytotoxic T-cell lines from dependence on IL-2[41]. These findings suggest

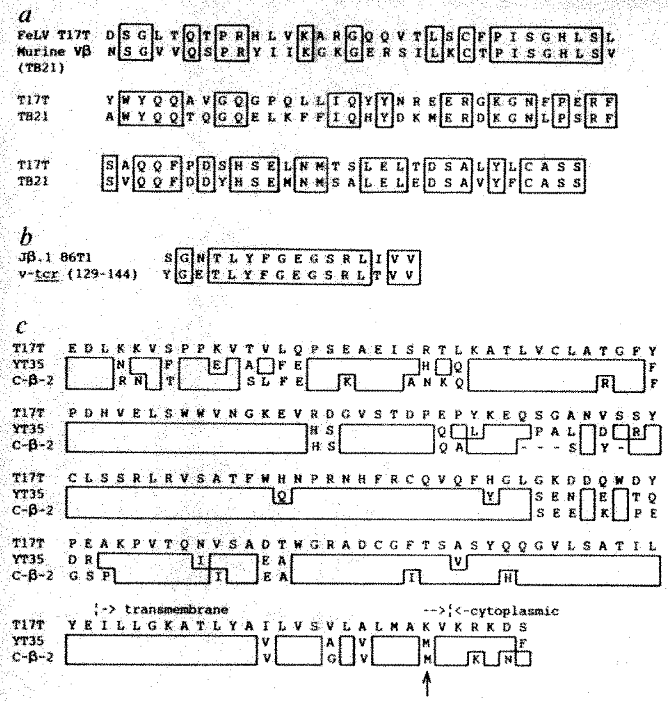
a
FeLV T17T D S G L T Q T P R H L V K A R G Q Q V T L S C F P I S G H L S L
Murine Vβ N S G V V Q S P R Y I I K G K G E R S I L K C T P I S G H L S V
(TB21)
T17T Y W Y Q Q A V G Q G P Q L L I Q Y Y N R E E R G K G N F P E R F
TB21 A W Y Q Q T Q G Q E L K F F I Q H Y D K M E R D K G N L P S R F
T17T S A Q Q F P D S H S E L N M T S L E L T D S A L Y L C A S S
TB21 S V Q Q F D D Y H S E M N M S A L E L E D S A V Y F C A S S
b
Jβ.1 86T1 S G N T L Y F G E G S R L I V V
v-tcr (129-144) Y G E T L Y F G E G S R L T V V
c
T17T E D L K K V S P P K V T V L Q P S E A E I S R T L K A T L V C L A T G F Y
YT35 N F E A F E H Q F
C-β-2 R N T S L F E K A N K Q R F
T17T P D H V E L S W W V N G K E V R D G V S T D P E P Y K E Q S G A N V S S Y
YT35 H S Q L P A L D R
C-β-2 H S Q A - - - S Y -
T17T C L S S R L R V S A T F W H N P R N H F R C Q V Q F H G L G K D D Q W D Y
YT35 Q Y S E N E T Q
C-β-2 S E E K P E
T17T P E A K P V T Q N V S A D T W G R A D C G F T S A S Y Q Q G V L S A T I L
YT35 D R I E A V
C-β-2 G S P I E A I H
¦-> transmembrane
-->¦<-cytoplasmic
T17T Y E I L L G K A T L Y A I L V S V L A L M A K V K R K D S
YT35 V A V M F
C-β-2 V G V M K N

Fig. 4 Alignment of FeLV T17T-22 v-*tcr* predicted amino-acid sequence with β-chain gene products. *a*, Murine V_β gene product[24] is aligned with the homologous region of v-*tcr* (residues 29–122): 57% of the amino acids can be matched allowing only single amino-acid insertions and deletions. If conservative amino-acid substitutions are scored as matches, identity is 69%. *b*, Comparison of v-*tcr* (129–144) with a murine J_β coding sequence; *c*, three-way comparison of representative human[33] and murine[32] C_β sequences with v-*tcr*. Open boxes, matches to v-*tcr*. Also indicated are the putative transmembrane and cytoplasmic domains of these gene products and a potentially important difference in the transmembrane region (arrow under sequence).

a possible cooperative role for v-*tcr* and v-*myc* in leukaemogenesis. We propose that the FeLV v-*tcr* gene in tumour T17 stimulates the antigen-receptor complex, and the FeLV v-*myc* gene augments the response or releases it from feedback regulation. Further studies will assess the possible oncogenic functions of v-*tcr*, *in vivo* and *in vitro*, alone and in combination with v-*myc*.

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Homozygotes for Huntington's disease

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Careful comparison of symptomatic individuals with normal controls has revealed the primary biochemical abnormality in many human genetic diseases, particularly recessive disorders[1]. This strategy has proved less successful for most human disorders which are not recessive, and where a single copy of the aberrant gene has clinically significant effects even though the normal gene product is present. An alternative approach that eliminates the impediment of a normal protein in affected individuals is to study homozygotes for the mutant allele[2]. For virtually all dominant human disorders in which homozygotes have been described, symptoms have been significantly more severe in the homozygote than in the heterozygote[3]. Thus, these disorders do not conform

to the classical definition of dominance which states that homozygotes and heterozygotes for a defect are phenotypically indistinguishable[3–5]. Instead, they display incomplete dominance, indicating that the normal allele may play a role in ameliorating the disease process. The *D4S10* locus, defined by the probe G8 and linked to the gene for Huntington's disease (HD), has permitted us to identify individuals with a high probability of being homozygous for this autosomal dominant neurodegenerative disorder[6–9]. These homozygotes do not differ in clinical expression or course from typical HD heterozygotes. HD appears to be the first human disease of genetically documented homozygosity that displays complete phenotypic dominance.

The HD gene was localized, in part, by study of the largest known HD family in the world, living along the shores of Lake Maracaibo, Venezuela[10–13]. The pedigree currently numbers almost 7,000 individuals: over 100 living symptomatic individuals and almost 900 family members born at 50% risk for carrying the gene defect but who are as yet asymptomatic. Among present generations of the pedigree, there are six unions between two symptomatic individuals, but there is nothing clinically unusual in their 31 offspring. We have concentrated our efforts on one large nuclear family, the 'H' family, in which both parents are living and many relatives could be assigned unequivocal genotypes.

To minimize the potential for bias in evaluating them, potentially homozygous offspring in the H family have been compared with 93 affected individuals, including 6 with juvenile onset of symptoms, and 307 'at-risk' relatives living in the same environment. Neurological, intellectual and behavioural function, as described in Fig. 1 have been assessed annually since 1981[14].

Six years ago 1 of 14 children was already mildly symptomatic and in 1985 a second member of the sibship was diagnosed with HD after displaying 'soft signs' for the previous five years. The age of onset and progression of symptoms in these two is typical compared to those in other HD patients in Venezuela and the United States[14,15]. In 1986, 7 siblings had soft signs and 5 remained neurologically normal.

The potential effects of homozygosity for the HD gene on intellectual function were also evaluated. A subset of the Venezuelan pedigree has been evaluated annually with a battery of neuropsychological tests assessing cognitive ability, memory and visual-motor dexterity[16–20]. The offspring of the H family have not shown any unusually precipitous decline on neuropsychological tests. The H family is also typical with respect to the behavioural disturbances which are often noted in HD, as assessed through structured and unstructured interviews[21–23].

The *D4S10* locus was originally defined by the G8 probe, a 17-kilobase (kb) fragment of genomic DNA that detects two polymorphic *Hin*dIII sites when hybridized to human genomic DNA[6,8]. A number of additional restriction fragment length polymorphisms (RFLPs) have been found using subclones of G8 or of R7, a second genomic clone overlapping G8[7,9]. The probes used in this study, along with the polymorphic sites and haplotypes they define, are shown in Fig. 2. Results from typing the H family for these sites are shown in Fig. 1*a* and *b*.

The HD gene usually segregates with the C1 haplotype at the *D4S10* locus in the Venezuelan pedigree. Reconstruction of the *D4S10* haplotypes of all 4 grandparents of the H sibship confirms that each H parent inherited the HD gene on a chromosome with the C1 haplotype. In addition, the mother received the A3 haplotype and the father the A5 haplotype from their respective unaffected parents. In the absence of recombination, their offspring homozygous for the HD gene would also be homozygous for the C1 haplotype at the *D4S10* locus.

All four possible *D4S10* genotypes are represented among the 14 children of the H family. Four received two copies of the C1 haplotype, suggesting they are homozygous at the HD locus. Six children received one copy of the C1 haplotype, making them probable heterozygotes for the HD gene, and the remaining 4 children did not inherit the C1 haplotype. This

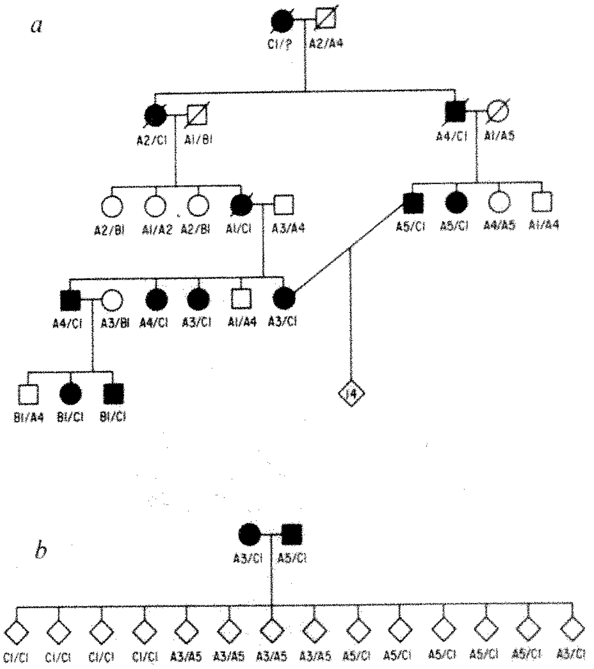


Fig. 1 Phenotypic assessment and segregation of *D4S10* and HD in the H family. *a*, Individuals from five generations of the H pedigree were typed for RFLPs at the *D4S10* locus. Family members diagnosed with HD are shown by darkened symbols and deceased individuals are denoted by a slash through the symbol. (Symbols are ○, female; □, male; ◇, one or more individuals, sexes not shown.) Haplotypes for *D4S10* were assigned as in Fig. 2. Genotypes were inferred from the phenotypic data after deducing the coupling phase of the various alleles for the *D4S10* RFLPs. In many cases, additional related individuals not shown here (for example, spouse and children) helped to confirm the assigned coupling phase. *b*, Inferred genotypes for the progeny of the HD by HD mating from *a*. The exact birth order of the children and the sex and HD status of each are not given to maintain confidentiality. Each C1/C1 individual has an approximately 92% probability of being homozygous for the genetic defect resulting in Huntington's disease.

Methods. The parents of the H family are second cousins once removed. The mother states that she is approximately 58 and her partner 63 years of age; no birth records exist. Each has been symptomatic for about 15 years. The mother began her pregnancies in her mid-teens with two miscarriages. In close succession, the couple had 15 children, including two sets of twins, one set of fraternal male twins and the other female. A female twin died at age 5 of gastroenteritis, leaving 14 living children available for study, ranging in age from 16 to 42 years. Blood samples from both parents, all 14 children and several other close relatives have been tested with more than 20 red-blood-cell and plasma markers and more than 50 polymorphic DNA markers to eliminate the possibility of non-paternity. Diagnosis of HD was based solely on the presence of characteristic extrapyramidal motor abnormalities. Behavioural disturbances were noted but were not themselves considered sufficient. The same neurologist (A.Y.) examined all members of the H family each year, joined in some years by between one and three other neurologists. Using the same rating scale, the neurologists demonstrated high inter-rater reliability in scoring[32]. The neurological examination assesses ocular motor ability, motor impersistence, fine motor coordination, tendon reflexes, and abnormal movements and postures such as chorea, dystonia and parkinsonian symptoms[14]. Functional capacity in patients was also rated[33]. About one-third of the at-risk individuals examined displayed soft signs, minor neurological irregularities in one or more of the areas assessed. A diagnosis of HD was only made when unequivocal signs were present. Diagnostic feedback is not given unless requested, which almost never happens. Research participants correctly understand that the study is long-range and that its goals are to learn the cause of the illness and to devise therapeutic interventions.

distribution of progeny genotypes is consistent with mendelian segregation, assuming equal viability. It differs significantly ($P < 0.01$) from the distribution expected if HD homozygotes die prenatally.

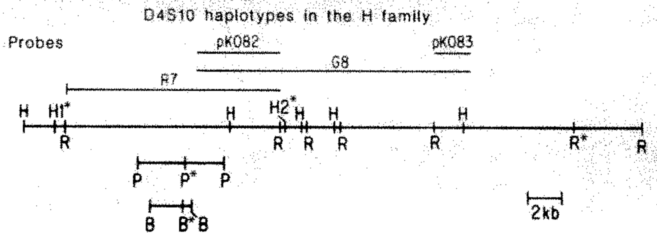
We cannot be absolutely certain that the 4 children homozygous for the C1 haplotype are homozygous for the HD gene because of the possibility of recombination in the gametes of either or both parents. The genetic distance between the two loci is ~4 centimorgans (4% recombination, 1 lod confidence interval = 1-8%). Consequently, each child homozygous for the C1 haplotype has an ~92% chance of being homozygous for HD. The probability that none of the 4 is homozygous for the defect is $(0.08)^4$ or 4×10^{-5} suggesting that homozygosity at the HD locus does exist in this family and is not lethal *in utero*.

We have chosen to omit exact correlations between the genotypes in Fig. 1*b* and the personal and neurological data on individual members of the sibship, but this does not affect the conclusions to be drawn from this study. First and foremost, we wish to protect the confidentiality of all family members, particularly those found to be homozygous. Of the 14 offspring, 10 are destined to become ill but 4 should remain normal. The fact that children of HD homozygotes are inevitably HD-gene carriers makes privacy all the more essential. A second reason for avoiding identification of the individual genotypes in this family is our desire to continue the prospective study with investigators in the field remaining blind to the genetic status of each subject.

Among the 4 potential homozygotes for the HD gene there is at least one representative of each of the three diagnostic categories: definitive HD, displaying soft signs, and neurologically normal. There appear to be no phenotypic differences, either in age of onset, symptoms, or progression of illness, between an affected probable HD homozygote and the typical HD heterozygotes from the Venezuelan kindred or any US sample of patients. Similarly, a potential HD homozygote who displays soft signs or is neurologically normal cannot be distinguished clinically from other at-risk individuals who are potential HD heterozygotes. In terms of the phenotypes currently being monitored by neurological and psychological examinations, HD displays complete dominance. This permits two conclusions concerning the motoric, cognitive and psychiatric symptoms of this disorder: first, they are not influenced by dosage of the defective gene; and second, they are not mitigated by the normal allele.

Matings between two heterozygotes for a dominant disease are so rare that the opportunity to study large numbers of progeny from such unions is exceptional[3,24-27]. In virtually every instance in which children are conceived between two heterozygotes, presumed homozygotes are quantitatively and sometimes qualitatively more severely affected than typical heterozygotes[3,24-27]. The only possible exception to this rule described previously involved a family with Best's disease, or hereditary macular degeneration[28], where unfortunately, homozygosity could not be confirmed genetically.

In view of the obvious dose effects displayed by putative homozygotes in other dominant diseases, our finding that homozygosity for the defective allele in HD does not result in a more severely affected phenotype than heterozygosity is surprising. The importance of this observation lies in the clues it may provide to the nature of the disease process and its primary lesion. Complete dominance is known to exist in *Drosophila* and is thought to be associated with mutations causing a 'gain of function'[29-31]. Clearly, HD cannot involve elimination of an essential enzyme activity as homozygosity is not lethal. The defect may compromise an enzyme activity that must exceed a strict threshold. Alternatively, the mutation might confer a novel property on a structural or regulatory protein, as the simple elimination of normal function would be expected to show a dosage effect or even lethality. If the HD defect causes a quantitative alteration, then overproduction of a normal protein, or the inappropriate production of a protein usually limited to a different tissue or to a different time of development, may be more likely than underproduction of a protein.



	H1*	B*	P*	H2*	R*
A1	–	–	–	+	–
A2	–	+	–	+	–
A3	–	–	–	+	+
A4	–	+	–	+	+
A5	–	–	+	+	+
B1	–	–	–	–	+
C1	+	+	–	+	–

Fig. 2 *D4S10* haplotypes in the H family. A map of the polymorphic sites (denoted by *) and the probes used to detect them is shown (above) with definition of *D4S10* haplotypes (below); G8 is an insert of single-copy human DNA, from the terminal region of the chromosome 4 short arm, cloned in the phage vector Charon 4A[6,7]. R7 is an overlapping genomic fragment also cloned in Charon 4A, and pK082 and pK083 are subclones of the indicated *Eco*RI fragments in pBR328. Two polymorphic *Hin*dIII (H) sites and one polymorphic *Pst*I (P) site were detected using pK082 as a probe. Single polymorphic sites with *Bgl*I (B) and *Eco*RI (R) were monitored by probing with R7 and pK083 respectively. **Methods.** For RFLP typing, genomic DNA from each individual was digested with the indicated enzyme (New England Biolabs), fractionated by agarose gel electrophoresis, transferred to a nylon membrane (Genatran, Plasco Inc.), and hybridized with ^{32}P-labelled probe DNA as described[6], except that probes were prepared by oligonucleotide priming[34]. Genomic DNA was obtained from permanent lymphoblastoid cell lines established for each member of the family as previously described[35,36]. The presence or absence of each site on a given chromosome was used to define haplotypes at the *D4S10* locus. When all sites were considered, 7 haplotypes were evident in this portion of the Venezuelan HD pedigree and the disease segregated with the C1 haplotype.

'Gene therapy' as a possible treatment for inherited disorders has engendered considerable interest recently. The normal allele at the HD locus does not apparently alter disease expression, so particularly imaginative approaches to gene therapy may be required for this condition: merely introducing a second normal allele is unlikely to have beneficial effects. Gene therapy aimed at blocking expression of the mutant allele (for example, by antisense mRNA) or at the introduction of unrelated gene(s) capable of correcting the biochemical defect may be a more rewarding approach.

The identification of specific genes or novel drugs capable of preventing the deleterious effects of HD is unlikely until the disease gene has been cloned and characterized. An intensive effort is underway to isolate the HD gene based on its map position despite limited knowledge of the nature of the defect. Cultured cells from HD homozygotes are very valuable in certain cloning strategies because their DNA contains no normal allele. Tissue samples from these individuals may ultimately enable identification of the gene product responsible for HD. Delineation of the molecular defect has obvious clinical importance and may also provide new insights into the biology of the nervous system. Of greater fundamental interest, perhaps, is that elucidating HD at the molecular level will reveal for the first time a genetic mechanism in humans capable of producing a completely dominant phenotype.

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Mesoderm induction in early *Xenopus* embryos by heparin-binding growth factors

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In early embryonic development the basic body plan arises because cells in different regions become programmed to follow different developmental pathways. We have proposed that in the early amphibian embryo this process of regional specification arises from the action of three different inducing factors, or morphogens (refs 1–5 and Fig. 1), but we have not until now had any idea of their chemical nature. In this paper we report (1) that pure basic fibroblast growth factor (bFGF), at very low concentrations and with high specificity, closely mimics the effect of the ventrovegetal (VV) signal and (2) that the transmission of the natural VV signal can be blocked by heparin, suggesting that it may be a heparin-binding factor such as bFGF.

In the main series of experiments, explants of ectoderm were cut from the animal pole region of stage 8 *Xenopus* blastulae (about 1,024–2,048 cell stage[6]) and exposed to bFGF. At stage 8 the explants include about 50–100 cells, but the cell number continues to increase by cleavage divisions without growth. Control explants round up over about two hours to form spheres with the original pigmented surface exterior and the original blastocoelic surface reduced to a small 'yolk plug'. Over the next two days, they become wrinkled and form epidermis around the outer surface. The internal cells may also all become epidermal but often some remain undifferentiated (Fig. 2*b*, *d*)[7,8]. Mesodermal cells are formed rarely, even then probably only because of occasional dissection errors which lead to inclusion of endodermal cells from the blastocoel floor. Treated explants also round up during the first two hours. At about 6 hours, when control embryos are middle gastrulae (stage $11–11\frac{1}{2}$), they elongate with the original yolk plug at one end. The degree of elongation varies between batches of embryos and can range from a slight pear-shape departure from sphericity, to a sausage shape about twice as long as it is wide (Fig. 2*a*). The process is reminiscent of the normal gastrulation movements on the ventral side of the embryo. After about 24 hours, when control embryos are at tailbud stages, the treated explants begin to swell by fluid uptake and become vesicles several times their original volume (Fig. 2*c*). Specimens were fixed for histological or immunohistochemical examination at about 64 hours of culture (control stage 41).

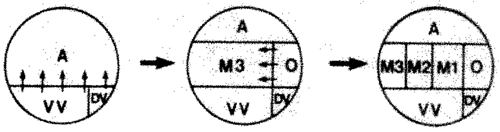

Fig. 1 Model of inductive interactions in the early amphibian embryo based on refs 1–5. The diagram depicts a blastula with the dorsal side to the right. Two mesoderm-inducing signals (DV and VV) are emitted from the vegetal hemisphere and induce an annulus of mesoderm consisting of a small organizer zone (O) and a large ventral mesoderm zone (M3). A dorsalizing signal emitted by the organizer then induces intermediate structures such as somites and kidney (M1, M2) from the ventral mesoderm zone. Abbreviations: A, animal hemisphere ('ectoderm'); DV, dorsovegetal; VV, ventrovegetal; O, organizer; M, mesoderm.

The appearance depends on the concentration of bFGF used. Between about 2 and 30 ng ml^{-1} the inductions closely resembled ventral-type mesoderms formed by explants from ventral or lateral marginal zones or by ventrovegetal-animal combinations[1,2,4,5]. They consist of a concentric arrangement of loose mesenchyme, mesothelium and blood cells within an epidermal jacket and sometimes contain a few muscle cells (Fig. 2*e*, *f*). The blood cells do not usually differentiate sufficiently to synthesize haemoglobin but have a characteristic morphological appearance. Between 30 and 120 ng ml^{-1}, most of the explants contain some muscle and significant blocks of it are often produced (Fig. 2*g*). Their identity was confirmed by

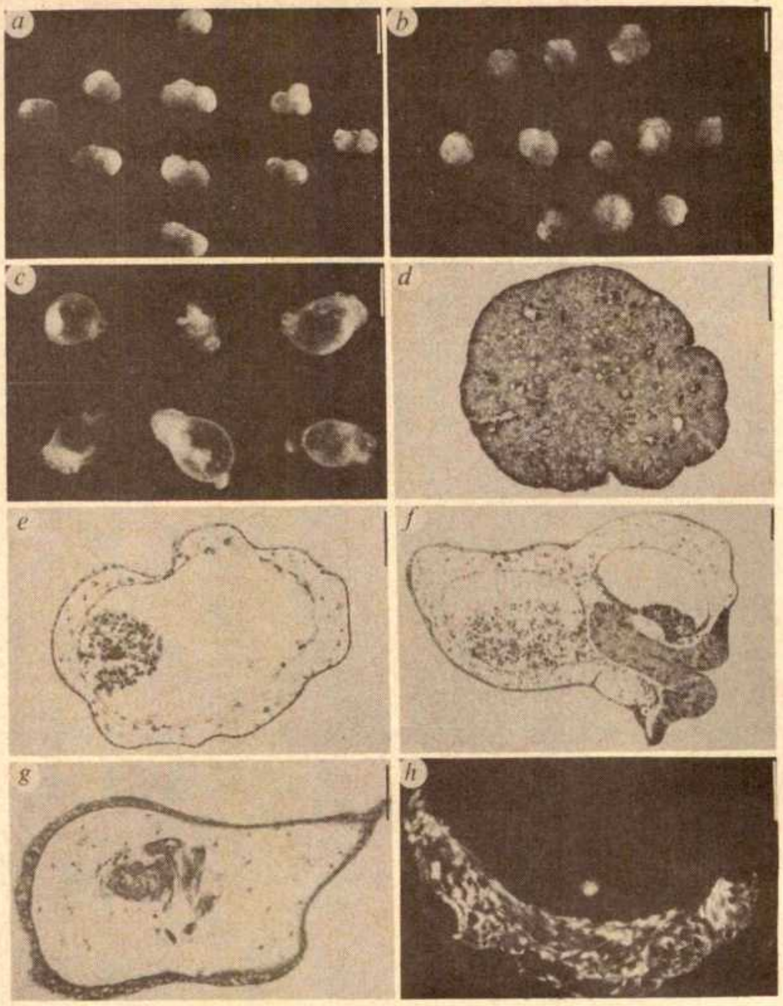

Fig. 2 Effect of bFGF on the development of ectodermal explants. Microoperative, histological and immunohistochemical methods as ref. 2. For stage series see ref. 6. *a*, Some bFGF-treated explants after overnight culture (control stage 20), showing elongation. *b*, Control explants after overnight culture. *c*, Some bFGF-treated explants after 3-day culture, showing vesicular forms. *d*, Section through 3-day control explant, which consists entirely of a solid mass of epidermis. *e*, Section through a bFGF induction, showing a concentric arrangement of epidermis, loose mesenchyme, mesothelium and blood cells. *f*, Section through ventrolateral explant from the marginal zone. These normally form ventral type mesoderm, and include also a region of yolky endodermal cells. *g*, Induction containing a significant muscle block, which arose from treatment with 120 ng ml^{-1} bFGF. *h*, Immunostaining of a muscle-containing induction with antibody to *Xenopus* myosin. Scale bars: *a–c*, 500 μm; *d–g*, 100 μm; *h*, 50 μm. Culture period for *c–h* was 3 days (control stage 41).

Methods. The bFGF used was purified from bovine brain by the method of Gospodarowicz and co-workers[22,23], followed by cation exchange chromatography on a Mono S column[17]. The mitogenic and mesoderm-inducing activities eluted together, associated with a broad OD peak between 0.4 and 0.5 M NaCl. The material from this peak was characterized as bFGF by the following methods. (1) On SDS-gel electrophoresis it appeared as a doublet with apparent relative molecular masses 16,500 and 18,000[22]. (2) On C3 reverse-phase chromatography it gave a major peak eluting at 30% acetonitrile which contained all the biological activity[17]. (3) Analysis of amino-acid composition of this peak gave values closely resembling those published for bFGF[22,23]. (4) Activity of a 60 ng ml^{-1} solution was blocked by a 1/240 dilution of an antibody prepared against a synthetic peptide representing the N-terminal 24 residues of bFGF[24], but was unaffected by various control sera. The Mono S fraction was not quite pure in that two minor peaks were revealed by the reverse-phase analysis. However, these had divergent amino-acid compositions and were without biological activity. The Mono S material was used for the biological experiments reported below as exposure to acid conditions on the reverse phase column causes a substantial reduction in specific activity. The bFGF concentrations quoted are based on the amino-acid composition analysis.

specific immunofluorescence with an antibody to *Xenopus* myosin (Fig. 2*h*). In general these muscle blocks are smaller than those found in explants from dorsolateral regions of the marginal zone, or in ventral marginal explants dorsalized by co-culture with organizer tissue[1,4,5]. The ventrolateral character of bFGF inductions provides strong support for our contention that there is a ventral inducing (VV) signal in the embryo, distinct from the organizer-inducing signal (DV) from the dorsovegetal quadrant[2,5].

A number of other growth factors were also tested for mesoderm-inducing activity using concentrations ranging from below physiological to 100 times physiological. No activity was found from any of the following: epidermal growth factor, platelet-derived growth factor (PDGF), insulin, chorionic gonadotrophin, interleukin-1 α and β, interleukin-3, interferon α and γ, tumour necrosis factor, transforming growth factor β, or the colony stimulating factors G-CSF and GM-CSF. No activity was found in various commercially available tissue culture media (with or without 10% fetal bovine serum), or in human urine. Activity was found for one other factor: embryonal carcinoma derived growth factor (ECDGF). This has been purified from the culture medium of PC13 murine embryonal carcinoma cells and is quite similar to bFGF in size and in heparin binding although it exhibits a slightly different biological specificity in terms of the spectrum of sensitive target cell types[9]. We have carried out fewer studies on it, but ECDGF appears similar to bFGF in its inductive effects, giving ventral inductions down to ~2 ng ml^{-1} and some muscle blocks at high concentrations ~100 ng ml^{-1}. Although we cannot prove that there are no other substances with similar activity the effect does seem to be fairly specific.

We also studied the concentration and time dependence of bFGF action. Concentration dependence was systematically investigated by exposing ectoderm explants to serial twofold dilutions (Fig. 3*a*). Individual explants show an all-or-none response although probably less than half the cells become mesodermal, so the percentage of explants forming vesicles at each concentration is shown. For most growth factors acting on cultured cells there is a range of partial responses over a concentration span of ~100-fold and assays are often based on a linear approximation to this partial response. The induction of mesoderm however follows a rather steeper dose–response curve with a less than 8-fold concentration range of partial responses. Whether this represents a genuine discontinuous (or threshold) response, as predicted for morphogen action[10] or whether it is simply a conventional but steep response is not clear at present. There are some sporadic inductions at the very low concentrations and the 50% response is found at about 2 ng ml^{-1} which is in the reported physiological range for mitogenic action of bFGF on fibroblasts[11]. Our own preparation was active down to about 50 pg ml^{-1} as a mitogen for murine 10T$\frac{1}{2}$ cells.

Several attempts were made to find the minimum necessary exposure time to obtain a response. Results were variable but suggested that whereas exposures of a few minutes were insufficient, ones of a few hours were sufficient (Fig. 3*b*). This agrees quite well with the estimate made by Gurdon *et al.*[12] that a minimum contact time of about 2.5 h between animal and vegetal explants is required to obtain an induction. The time

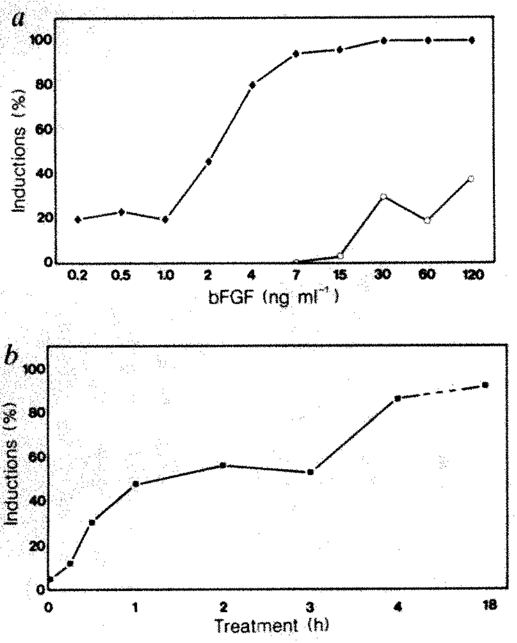

Fig. 3 *a*, Dose-response curve for induction by bFGF. Explants were cultured in Terasaki plates in a final volume of 12 μl of half strength Normal Amphibian Medium (NAM)[2] plus 1 mg ml^{-1} bovine serum albumin (BSA), over 2 μl of agarose to prevent adhesion to the plastic. Various concentrations of bFGF were present continuously in the wells. Each point represents tests on about 30 explants. Diamonds, percentage of induced explants scored visually as large vesicles; circles, those which proved after sectioning to contain significant muscle blocks such as those in Fig. 2*g*, *h*. *b*, Effect of withdrawing bFGF at various times. Design as in *a*, except that explants were exposed to bFGF at suprathreshold concentration for various times then removed from the wells, rinsed, and replaced in fresh wells with NAM/2 and 1 mg ml^{-1} BSA. The total number of explants used was 199.

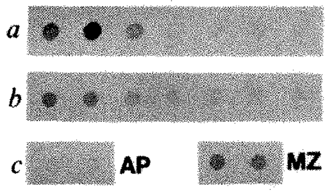

Fig. 4 Detection of muscle in FGF inductions by a dot blot method, using the same antibody to *Xenopus* myosin as in Fig. 2*h*. *a*, Explants treated with twofold dilutions of bFGF starting at 60 ng ml^{-1}. The first four became vesicles and the first three are positive for muscle. *b*, Explants all treated with 60 ng ml^{-1} bFGF but in the presence of heparin at concentrations of 0, 0.01, 0.1, 1, 10, 100 and 1,000 μg ml^{-1}. The first five explants formed vesicles and the blots show the progressive inhibition of muscle formation. *c*, Positive and negative controls are, respectively, marginal zone (MZ) and ectoderm (AP) explants cultured for 3 days.

Methods. After culture, explants were homogenized in 0.12 ml, 50 mM, TrisCl *p*H 7.4, 1 mM EDTA, 1% sodium deoxycholate, 1 mM phenylmethylsulphonylfluoride (PMSF), 100 nM pepstatin A. This was centrifuged for 4 min at 8,700*g* to remove pigment granules. The samples were applied to nitrocellulose membrane using a Biorad dot blot apparatus. The membrane was removed and treated as follows. (1) With 3% BSA + 5% goat serum in Dulbecco's phosphate-buffered saline A (PBSA), 1 h 37 °C (or overnight 4 °C). (2) Anti-myosin serum 1/200 in PBSA, 2 h room temperature. (3) Several washes in PBSA over 45 min. (4) Add 1/1,000 goat anti-rabbit, affinity-purified horseradish peroxidase (HRP) conjugate (Miles) in 3% BSA 1% goat serum in PBSA, 2 h room temperature. (5) Washed as before and rinsed in water. (6) Add 0.5 mg ml^{-1} diaminobenzidine, 0.025% cobalt chloride, 0.02% ammonium nickel sulphate, 0.13% H_2O_2 (100 vol.) for about 1 min then rinsed several times in water.

Table 1 Blockage of endogenous mesoderm-including signal by heparin

Combination	Conditions	Positive	Total
AP-VP	No additives	11	14
AP-AP	No additives	0	12
AP-VP	Heparin (1,000 μg ml^{-1})	1	7
AP-VP	Heparin (100 μg ml^{-1})	0	11
AP-VP	Heparin (10 μg ml^{-1})	5	7
AP-VP	Chondroitin sulphate (100 μg ml^{-1})	11	12
AP-VP	No gap, Heparin (1,000 μg ml^{-1})	8	10

AP, animal pole explant; VP, vegetal pole explant. Explants were left in the transfilter apparatus overnight (~16 h) then removed and cultured separately for two more days. Inductions were scored visually by the formation of large vesicles, and were then sectioned. All vesicles indeed contained mesoderm, 75% of a ventral type and the remainder dorsal or intermediate types[5]. The no gap group were combinations assembled in the presence of heparin in an agar-coated dish. Heparin was grade I or grade II from Sigma; chondroitin sulphate from Seikagaku Kogyo Co. Ltd.

requirement is also reminiscent of that for induction of DNA synthesis by PDGF[13,14]. Like PDGF, bFGF leads to the activation of protein kinase C[15], but treatments of ectoderm explants with cAMP, cGMP, A23187 and phorbol 12-myristate-13-acetate, which might be expected to mimic the various second message effects, have so far all proved to have no effect.

The best known effect of bFGF on cultured cells is to stimulate entry into S phase, so we were interested in whether there was any stimulation of ^{3}H-thymidine incorporation in treated explants. Incorporation into batches of explants, treated or untreated, was measured for three two-hour periods between stage 8 and 12, by which time elongation has begun. No stimulation of incorporation was observed and we conclude that the inductive effect is not secondary to growth stimulation. In many respects this is not surprising because cell division is synchronous in *Xenopus* embryos and occurs every 30 min at 25 °C up to the mid-blastula transition (~4,096 cells)[16], and although synchrony breaks down after this there are no systematic regional differences in cell division until after tissues have begun to differentiate during neurulation.

Both bFGF and ECDGF are members of a family of growth factors that bind tightly and specifically to heparin[17]. Heparin has been reported to inhibit the binding of bFGF to its receptor[18] and we have found that addition of heparin reduces the effectiveness of the bFGF as an inductor. As the heparin concentration is increased in the presence of a high concentration of bFGF, the production of muscle is lost (Fig. 4) and then the induction is suppressed altogether. This repression occurs at substantial molar excesses of heparin, so it seems likely that the mechanism involves a competition for the bFGF between the heparin in the bulk solution and binding sites within the cell mass. It is not necessary to assume that the heparin–bFGF complex is itself inactive.

This effect suggested a way of finding whether the endogenous factor(s) are, like bFGF and ECDGF, heparin-binding factors. It is known that mesoderm inductions, mainly of a ventral character, can be obtained if animal and vegetal explants are cultured on either side of an 0.4-μm Nucleopore filter[19], showing that the effect is due to diffusible factor(s). A sufficiently large concentration of heparin in the space between the explants might intercept the signal. Transfilter experiments were set up with the explants separated by a nylon gauze about 0.1 mm thick with holes about 0.1 mm square instead of by a Nucleopore filter. This holds the explants apart and allows access of reagents from the side.

The results are shown in Table 1. Controls in which there were no additives, or in which another glycosaminoglycan, chondroitin sulphate, was included, gave a high frequency of positive inductions. However with heparin at $\geq 100\,\mu g\,ml^{-1}$ there was only a single positive. In separate experiments it was shown that heparin at $1\,mg\,ml^{-1}$ has no toxic effects, and no inductive effects on isolated ectoderms. It does not inhibit the formation of mesoderm in animal-vegetal combinations or in marginal zone explants, showing that its presence in solution between the inducing and responding tissues is necessary for blocking.

We cannot, of course, exclude other possible reasons for the blocking action of heparin. But in view of the various controls we feel that the most likely explanation is that the natural ventral mesoderm-inducing factor, in the embryo, is a heparin-binding factor, and that the heparin in the gap can intercept it and prevent it from reaching the target tissue.

In addition to these specific results this work suggests two general conclusions. First, mesoderm induction is a highly specific process, unleashed by a small group of regulatory proteins. This is in sharp contrast to neural induction which can be evoked by a wide range of substances[20,21]. Second, it suggests a new type of role for growth factors in early development: not as mitogens but as morphogens that control the developmental pathways selected by cells.

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Note added in proof: A third heparin-binding growth factor, acidic FGF (provided by Dr D. Gospodarowicz), is also active in inducing mesoderm.

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Suppression of *in vitro* haematopoiesis following human immunodeficiency virus infection

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Viral infections are frequently associated with haematological disorders (reviewed in ref. 1). Abnormalities including leukopenia, anaemia and thrombocytopenia are commonly observed in patients with the acquired immune deficiency syndrome (AIDS) or the AIDS-related complex (ARC)[2–12]. The underlying cause of these haematological abnormalities is poorly understood. We report here that bone marrow progenitors isolated from AIDS or ARC patients are responsive to recombinant human granulocyte-macrophage colony stimulating factor (rGM-CSF) and recombinant erythropoietin. Antibodies present in the serum of patients infected with the human immunodeficiency virus (HIV), however, could suppress the growth of these progenitors, but not the growth of progenitors from HIV seronegative controls. A component of this immune-mediated suppression appears to be antibodies directed towards the envelope glycoprotein (gp120) of HIV.

To study the effects of rGM-CSF and erythropoietin on human progenitor cells derived from patients with AIDS or ARC, disorders due to infection with HIV[13,14], adherence-depleted bone marrow mononuclear cells from untreated AIDS and ARC patients as well as healthy, HIV seronegative donors were cultured *in vitro*. At various concentrations of GM-CSF, the number, rate of development, size and morphology of the granulocyte-macrophage (CFU-GM), early erythroid (BFU-E), or multi-lineage (CFU-Mix) colonies derived from AIDS or ARC bone marrow generally did not differ from that of healthy HIV seronegative donors in the presence or absence of HIV seronegative serum (Fig. 1). In the presence of sera from HIV seropositive patients, however, CFU-GM and BFU-E development was markedly suppressed in bone marrow progenitor cultures from patients with AIDS or ARC, but not in marrow cultures from healthy, HIV seronegative donors (Fig. 1). The suppression by the HIV seropositive serum samples tested ranged from 50 to 90% inhibition in the numbers of CFU-GM or BFU-E colonies observed using different AIDS or ARC bone marrow donors. Suppression was seen in the cultures of bone marrow cells from all the eight AIDS or ARC donors tested but not in any of the cultures of marrow cells from normal donors. Because of the low numbers of CFU-Mix normally observed in cultures established from bone marrow cells from healthy donors or AIDS or ARC patients, suppression of CFU-Mix could not be demonstrated by the addition of HIV seropositive sera.

To investigate further the suppressive effect of serum on CFU-GM and BFU-E colonies derived from AIDS or ARC bone marrow, serum samples obtained from patients before and after they had become positive for HIV antibody were tested for their ability to inhibit AIDS or ARC bone marrow colony formation. There was no effect of the preimmune serum on CFU-GM or BFU-E colony formation, but upon seroconversion the serum markedly suppressed *in vitro* myelopoiesis and erythropoiesis (Fig. 2). This finding was observed in all three AIDS or ARC bone marrows tested. Bone marrows taken from healthy, HIV seronegative donors did not demonstrate any suppression with either the preimmune or HIV seropositive serum (data not shown). Nonspecific effects of serum on allogenic cells (such as, for example, anti-HLA antibodies, anti-blood group antibodies) were not apparent in our study as normal sera did not have any suppressive effect on bone marrow progenitors from individuals with differing ABO blood types. Similarly, preimmune sera did not suppress CFU-GM or BFU-E colony formation in AIDS or ARC bone marrow progenitor cultures but the

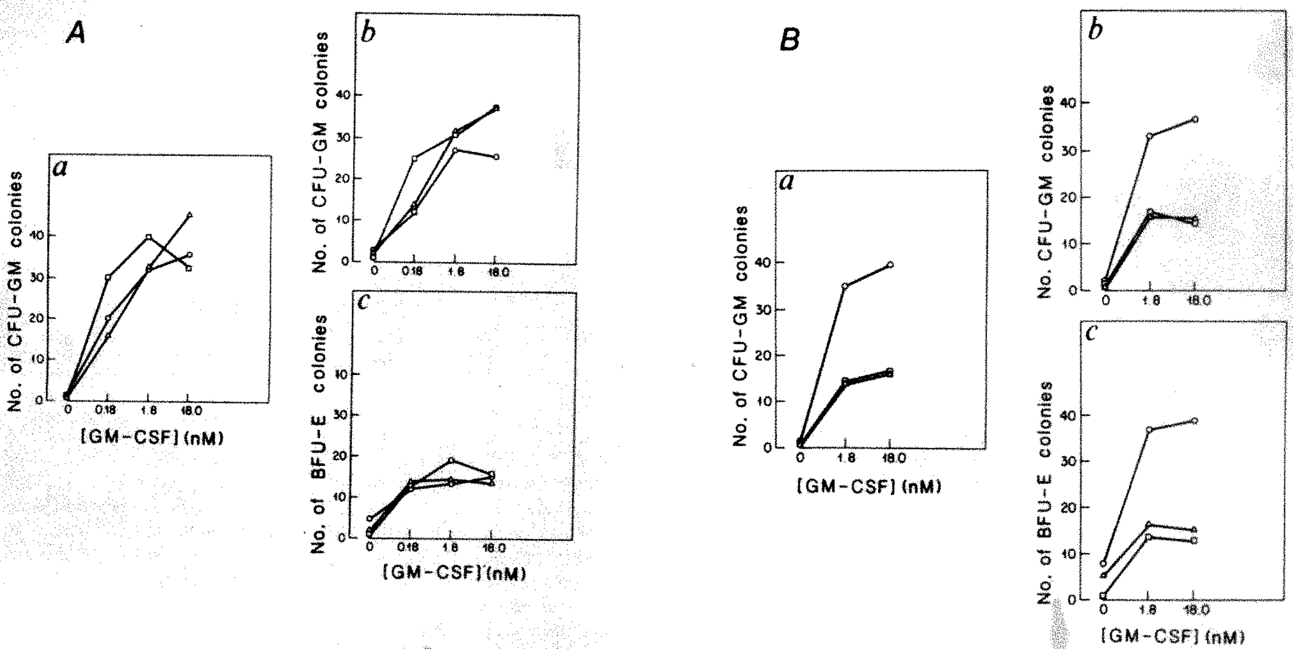

Fig. 1 Effect of sera from HIV-infected patients on GM-CSF-dependent colony formation by bone marrow progenitors from a healthy, HIV-seronegative donor (*A*) or an HIV-infected donor (*B*). Adherence-depleted bone marrow progenitor cells were plated in methylcellulose at the indicated concentrations of rGM-CSF in the presence of serum samples from patients with AIDS (△), ARC (□), or from HIV seronegative control subjects (○). The numbers of granulocyte and/or macrophage (*a*, *b*) or erythroid (*c*) colonies per 5×10^4 cells were scored either in the absence (*a*) or presence (*b*, *c*) of recombinant erythropoietin. Each point is the mean of duplicate plates.

Methods. Bone marrow aspirates were taken, after obtaining informed consent, from healthy volunteers unexposed to HIV or from patients with AIDS or ARC who fulfilled the diagnostic criteria for these disorders established by the Centers for Disease Control and the National Institutes of Health. The AIDS or ARC patients neither had active infections involving opportunistic pathogens nor were receiving any potentially myelosuppressive antibodies, cytotoxic chemotherapy, or other medications. Pathologically, myelodysplasia and erythrodysplasia with maturational abnormalities were noted in the bone marrow biopsies of the AIDS or ARC patients. The bone marrow was aspirated from the posterior iliac crest using standard techniques. The marrow was aspirated into preservative-free herapin (Sigma) and low-density, adherent-depleted cells were obtained by methods previously described[37]. *In vitro* culture techniques for myeloid and erythroid progenitor cells were as described[37]. Human sera were added to appropriate plates at a final concentration of 5% (v/v). Sera were tested for HIV antibodies by an enzyme-linked immunosorbent assay (Electro-Nucleonics Inc). The rGM-CSF expressed by CHO cells was purified as described[38,39] and diluted with Iscove's Modified Dulbecco's Medium (IMDM) so that the protein concentration was 50 μg ml^{-1}. This solution was diluted further in IMDM so that the final dilution in the culture ranged from $1:10^2$ to $1:10^5$ (18–0.018 nM). In those cultures which required the addition of erythropoietin, one unit of highly purified recombinant erythropoietin[40] derived from CHO cells was added dropwise to each of the cultures three days after the start of incubation. Granulocyte and/or macrophage (CFU-GM), early erythroid (BFU-E), and mixed-lineage (CFU-Mix) colonies were scored on day 12–14. Four additional normal bone marrows and seven other AIDS or ARC bone marrows tested showed similar results to the one presented here.

post-immune, HIV seropositive sera did. The myelosuppressive effect was, therefore, only seen in the HIV seropositive serum samples on bone marrow from AIDS or ARC patients.

In preliminary studies, we have been able to isolate HIV from pooled bone marrow progenitor colonies grown *in vitro* from an AIDS patient and were able to infect adherence-depleted bone marrow taken from a HIV seronegative donor (Table 1). Virus, however, could not be isolated from the pooled progenitors of three other AIDS patients. This failure may be explained by the observation that GM-CSF can inhibit HIV production from cells of myeloid origin, such has been shown for the monocyte cell line U937[15], or explained by the variability of HIV recovery from infected hosts using available culture techniques. These observations suggest that progenitors and/or terminally differentiated cells in the cultures may be infected with HIV. HIV infection of the host may not be directly cytopathic to bone marrow progenitors because, in the absence of anti-HIV antibodies, progenitor proliferation occurs normally *in vitro* in the presence of purified rGM-CSF (Fig. 1). Still other progenitor cells may be refractory to infection because, even in the presence of HIV seropositive serum, colony formation is not completely inhibited. Recently, we (J. Sullivan, J.E.G. *et al.*, unpublished observations), as well as others[16], could detect virus infection of myeloid precursors in bone marrow from AIDS patients by *in situ* hybridization. Similarly, follicular dendritic cells and monocyte-macrophages, the progeny of CFU-GM or CFU-M, have been shown to be infected with HIV *in vivo*[17–23] and monocyte-macrophages and established cell lines of human B cells, monocytes, myeloblasts and promyelocytes could be infected with HIV *in vitro*[15,21–26]. These cells do not demonstrate gross cytopathic effects or impaired proliferation.

One hypothesis to explain the suppressive effects of sera is that bone marrow progenitor cells are infected by HIV and those infected cells may have HIV antigens such as the major envelope glycoprotein, gp120, or the transmembrane protein, p41, expressed on the cell surface. Antibodies to retroviral antigens might inhibit the proliferation of the virally infected progenitor cells directly or indirectly through an antibody/dependent mechanism such as antibody-dependent cell-mediated cytoxicity (ADCC). To test directly whether the suppressive activity found in sera from HIV-infected patients is due to anti-HIV antibody, immunoglobulin from a patient with AIDS was purified and added at several concentrations to bone marrow progenitors from an ARC patient (Fig. 3). When compared to purified pooled control immunoglobulin, the purified AIDS immunoglobulin showed significant suppression of haematopoietic colony formation. These observations have been extended in experiments using rabbit heteroantiserum directed against recombinant gp130 which has previously been shown to neutralize HTLV-3b infection of H9 cells[27]. Using the rabbit's preimmune serum and heteroantiserum against recombinant gp130 at a final concentration of 12.5 μg ml^{-1}, we found in duplicate cultures a decrease in CFU-GM from a mean of 18.5 colonies per 5×10^4 cells to a mean of 8.5 colonies per 5×10^4 cells. This observation suggests

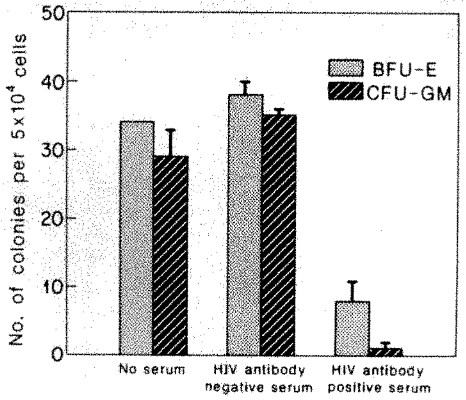

Fig. 2 Appearance of haematopoietic-suppressive activity in serum from a patient following HIV seroconversion. Adherence-depleted bone marrow progenitor cells from a patient with AIDS were plated in the presence of rGM-CSF (1.8 nM) and recombinant erythropoietin (1 U ml^{-1}) in the presence of serum from a patient before and after apparent infection by HIV. Each individual point represents the mean colony count (±s.d.) of duplicate plates. The preimmune serum which was HIV seronegative was drawn more than six months before the patient was found to be HIV seropositive. Culture methods as in Fig. 1. Marrow samples from two additional AIDS or ARC patients have also been tested with these serum samples and both gave results similar to the results presented.

Table 1 Reverse transcriptase activity in bone marrow cultures

	Day 7	Day 11	Day 14	Day 19	Day 21
Bone marrow from AIDS patient (WS)	5.13	5.27	4.09	1.49	0.49
Normal bone marrow infected with control supernatant (+epo)	0.00	0.00	0.00	0.00	0.00
Normal bone marrow infected with HTLV-IIIb (+epo)	0.61	6.44	7.30	6.01	3.16
Normal bone marrow infected with control supernatant (−epo)	0.00	0.00	0.00	0.00	0.00
Normal bone marrow infected with HTLV-IIIb (−epo)	0.00	0.00	0.69	1.08	1.05

Reverse transcriptase activity is given as c.p.m. × 10^5. Bone marrow was grown in culture for 12–14 days as in Fig. 1. The cultures were then pooled, washed in RPMI plus 20% fetal calf serum (FCS) and 0.2% hexadimethrine bromide (Sigma) and co-cultivated with 10^6 peripheral blood mononuclear cells from a HIV seronegative donor in the presence of 20 μg phytohaemagglutinin (Sigma) and 10% interleukin-2 (Cellular Products). Supernatants were collected at various time intervals and assayed for reverse transcriptase activity as described[27]. The normal bone marrow was inoculated either with the HTLV-IIIb isolate of HIV or the control supernatant from uninfected H9 cells for 2 h, after which the adherent cells were depleted and the cultures established from the low-density population. The normal bone marrow progenitors were grown in the presence or absence of erythropoietin (epo); the cultures were pooled and co-cultivated for recovery of HIV as for the AIDS bone marrow.

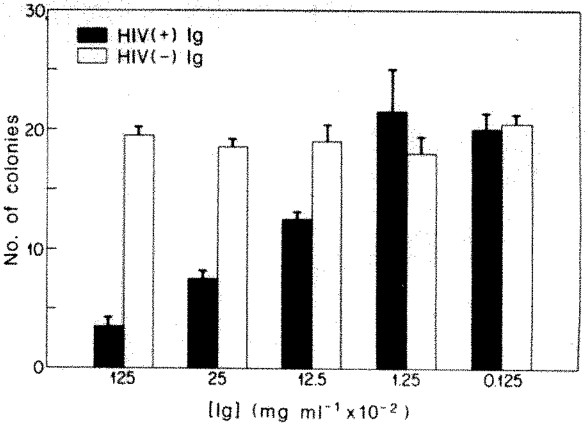

Fig. 3 Inhibition of GM-CSF-dependent colony formation of bone marrow cells from an HIV-infected patient by purified immunoglobulin from an AIDS patient. Adherence-depleted bone marrow progenitor cells from an ARC patient were plated in methylcellulose cultures in the presence of rGM-CSF and the indicated amounts of purified immunoglobulin from an AIDS patient (■) or from a pool of sera from normal donors (□). Each colony count represents the mean (±s.d.) of duplicate determinations.

Methods. The immunoglobulin fraction of serum from an AIDS patient was obtained by ammonium sulphate precipitation followed by elution from a protein A-Sepharose (Pharmacia) column as described[27]. Protein concentration by amino-acid analysis was 25 mg ml^{-1}. The normal human immunoglobulin G (IgG) used (Cappel) was shown to be negative for HIV antibodies by an enzyme-linked immunosorbent assay (Electro-Nucleonics Inc.). The normal human immunoglobulin was resuspended in phosphate-buffered saline to a concentration of 25 mg ml^{-1}. These immunoglobulins were diluted in IMDM to the final concentrations shown. Methods for establishing the cultures were as in Fig. 1, except that recombinant erythropoietin was not added to these cultures. GM-CSF was added at a final concentration of 1.8 nM. No colony growth was observed in the absence of GM-CSF.

that antibody directed against the major envelope glycoprotein of HIV contributes to the myelosuppression observed *in vitro*. From our initial studies, we suggest that the progenitor cells may be infected with HIV and are not grossly perturbed in their proliferative potential unless antibody to HIV is present.

In other disease conditions associated with peripheral cytopenias, serum is a potent source of a variety of colony-stimulating activities[28-30]. For example, we have found that sera from many patients with aplastic anaemia can readily support colony formation by normal bone marrow progenitor cells in the absence of additional rGM-CSF (R.E.D., J. M. Rappeport and D. G. Nathan, unpublished observations). In contrast, none of the serum samples from either AIDS or ARC patients, despite their haematologic abnormalities, had any colony-stimulating activity as measured in our assay system with normal bone marrow target cells. This implies that there is a defect in haematopoietic growth factor production in HIV-infected patients. Others[31] have reported defects in lymphokine production by T lymphocytes from AIDS patients which could account for some of the depression of colony-stimulating activity found in the sera from these individuals. Still others have reported the production of soluble suppressor factors from the peripheral blood mononuclear cells of AIDS or ARC patients[32,33]. It will also be interesting to examine production of haematopoietins by HIV-infected monocytes because these cells are known to produce colony-stimulating activity[34] as well as other proteins including interleukin-1[35], which has recently been demonstrated to activate the expression of GM-CSF[36]. The inability of the virally infected T cells and monocytes to produce factors regulating the production of haematopoietic cells in combination with the haematopoietic suppressive effect of antibodies directed against HIV would certainly contribute to the common occurrence of cytopenias in AIDS and ARC patients.

The finding that sera from HIV-infected patients are capable of inhibiting GM-CSF-dependent colony formation by bone marrow progenitor cells in AIDS and ARC patients potentially complicates the use of hematopoietins in AIDS patients. The suppression of GM-CSF-dependent colony formation by the sera of AIDS patients, however, was never complete and the results obtained in an immunodeficient, pancytopenic rhesus monkey (*Macaca mulatta*) naturally infected by a Type D retrovirus[37] indicate that administration of this growth factor may be effective in stimulating haematopoiesis in AIDS patients. We expect that clinical studies of the effectiveness of GM-CSF in overcoming the virus-induced cytopaenias in AIDS patients as well as further characterization of the HIV-induced haematopoietic suppressive activity to provide insight into the pathophysiology of haematopoiesis following retroviral infection in man.

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Localization of clathrin light-chain sequences mediating heavy-chain binding and coated vesicle diversity

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At least four distinct forms of clathrin light chains are found in mammalian cells[1–5]. This molecular variability derives from tissue-specific patterns of expression of LC_a and LC_b genes[6]. Sequence analysis shows an overall homology of 60% between LC_a and LC_b and the presence of brain-specific insertion sequences. These findings suggest that the different light chains have both shared and specialized functions[6]. To address this question we have used a panel of monoclonal antibodies to identify two structurally and functionally distinct regions in the clathrin light-chain sequences. One region (residues 158–208) is exposed in native clathrin structures (triskelions[7,8] and coated vesicles) and includes the brain-specific insertion sequences. The second region (residues 93–157), which is cryptic in native clathrin structures, is involved in binding the clathrin heavy chain and contains the region of strongest homology with intermediate filament proteins[6].

A shared feature of all four light chains is their binding to the clathrin heavy chain. We reasoned that sites involved in heavy-chain binding should be accessible in free light chains but not in triskelions or coated vesicles. These were identified using a panel of 18 monoclonal antibodies raised against separated light chains. Figure 1 shows the specificity of these antibodies for brain and non-brain forms of LC_a and LC_b in a solid-phase radioimmunoassay and their binding to coated vesicles in a co-sedimentation assay. Eleven of the anti-light-chain antibodies bound as well to coated vesicles as the X22 antibody which recognizes an exposed determinant of the clathrin heavy chain[9,10]. The other seven antibodies gave weak or no binding to coated vesicles although they react strongly with free light chains. In addition, this second group of antibodies shows no reactivity with purified clathrin triskelions as measured by immunoprecipitation, although they do immunoprecipitate free light chains. In contrast, all but two of the antibodies (LCB.72,74) which bind to coated vesicles are effective in immunoprecipitation of triskelions. Thus light-chain determinants become cryptic as a result of light chain–heavy chain association within the clathrin triskelion rather than from triskelion assembly into coated vesicles.

Seven of the antibodies tested react exclusively with the brain forms of the clathrin light chains. All show preferential binding to LC_b, four are LC_b-specific and three react weakly with LC_a. The specificity of these antibodies suggests they are directed against sites involving the brain-specific insertion sequences. As five of these antibodies react in Western blots, presumably recognizing linear determinants, it is likely that they interact directly with residues in the insertion sequences. This is confirmed by the peptide-binding studies described here. A critical finding is that all seven of these tissue-specific antibodies bind to sites that remain exposed when clathrin light chains are associated with triskelions or coated vesicles. This indicates that the brain-specific sequences are accessible in the *in vivo*, functional forms of clathrin. It is therefore possible that, in the brain, these sequences provide sites of interaction with other proteins involved in brain-specific functions of coated vesicles.

A second method was used to identify light-chain sites involved in interactions with the clathrin heavy chain. This method measures the capacity of monoclonal antibodies to inhibit the

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	LC specificity			
	Brain		Adrenal	
Antibody	LC_a	LC_b	LC_a	LC_b
Control	-	-	-	-
X43	+	+	+	+
X49	+	+	+	+
X50	+	+	+	+
X16	+	-	+	-
X44	-	+	-	+
X45	-	+	-	+
X46	-	+	-	+
CVC.1	+	-	+	-
CVC.6	+	-	+	-
CVC.7	+	-	+	-
LCB.1	(+)	+	+	+
LCB.2	(+)	+	-	-
LCB.4	(+)	+	-	-
LCB.3	-	+	-	-
LCB.5	(+)	+	-	-
LCB.6	-	+	-	-
LCB.72	-	+	-	-
LCB.74	-	+	-	-
X22	-	-	-	-

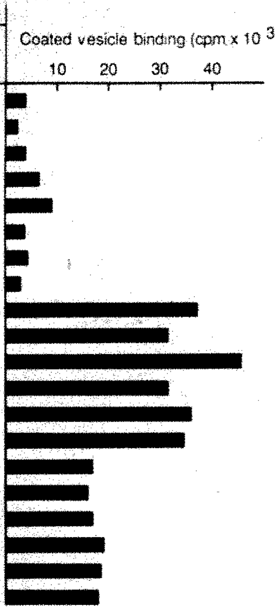

Fig. 1 Binding properties of anti-light-chain antibodies. Monoclonal antibodies reacting with purified clathrin light chains were tested for binding to bovine brain coated vesicles by co-sedimentation. The specificity of each antibody for separated LC_a and LC_b light chains (LC) derived from brain and adrenal clathrin is indicated to the left of the vesicle-binding data: +, strong reactivity; (+), weak reactivity; −, no reactivity.

Methods. Monoclonal antibodies were produced and isolated according to established procedures[14] by fusion of mouse popliteal lymphocytes or splenocytes with the SP2/0 cell line[15] and rat popliteal lymphocytes with the YB2/0 cell line[16]. Antibodies used in all studies were purified from ascites fluid of mice or rats[17]. X43–X50 were derived from rats (Lou/M) immunized with purified LC_b in footpads[9]. LCB.1–LCB.4 were derived from mice (BALB/c) immunized with bovine brain LC_b intraperitoneal and triskelions intravenous[18]. LCB.5–LCB.74 were derived from mice (BALB/c) immunized with brain LC_b C.1 peptide (amino acids 158–228) in footpads[9]. Production of CVC.1–CVC.7[11], X16 and X22[9] was as reported. Antibody specificity for purified light chains was determined by indirect solid-phase radioimmunassay[9]. Antibody binding to light chains, immobilized on a polyvinyl chloride plate, was detected using iodinated $F(ab')_2$ fragment of rabbit anti-mouse Ig (^{125}I-RAM). To obtain clathrin light chains, coated vesicles were isolated from bovine brain or adrenal tissue[19], suspended in 10 mM Tris, 0.2 mM EDTA, *p*H 7.5 and exposed to 100 °C for 10 min. After centrifugation, LC_a and LC_b were purified from the supernatant by DEAE chromatography[2]. The LC_a pool was depleted of residual LC_b, and the LC_b pool depleted of residual LC_a using affinity columns of X45-Sepharose 4B and X16-Sepharose 4B respectively[9]. Coated vesicles (CV) for cosedimentation were prepared from bovine brains[19] and suspended at 100 mg ml^{-1} in 100 mM MES, 2.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, *p*H 6.5 (buffer A') with 0.02% NaN_3. To assay antibody binding to coated vesicles, 1 mg of CV was combined with 5 µg purified monoclonal antibody in 100 µl of buffer A' with 0.5% bovine serum albumin (buffer A'-BSA) incubated 1 h, 4 °C then washed twice with 1 ml buffer A'-BSA by centrifugation for 10 min at 13,000*g*. The ^{125}I-RAM (100,000 c.p.m.) in 10 µl was incubated with the CV pellet in 100 µl buffer A'-BSA, 1 h, 4 °C, then washed twice, as before. CV pellets were counted for radioactivity bound: control binding (background) was determined using 5 µg of anti-Leu 10 a monoclonal antibody recognizing a human cell surface antigen. X22 reacts with clathrin heavy chain[9].

reassociation of separated light chains with heavy chains. Only three light-chain-specific antibodies (X16, X43, X45) gave a significant inhibition of subunit reassociation, and all are directed against sites that become cryptic on association with the heavy chain. Similar inhibition of light chain–heavy chain association was seen with either purified immunoglobulin G (IgG) or monovalent antigen-binding fragments (Fab) fragments (Fig. 2) thus ruling out artefactual effects caused by aggregation due to antibody bivalency. Furthermore, inhibitory effects were observed whether light chains were reassociating with heavy chains derived from free or assembled triskelions. The inhibitory effects correlated with the light-chain specificity of the antibodies. Fab fragments of the anti-LC_a antibody X16 strongly inhibited LC_a association with heavy chain but did not affect LC_b association: those from the anti-LC_b antibody X45 had the reverse effect. Fab fragments of X43, which binds more strongly to LC_b than LC_a, had a detectable inhibitory effect only on LC_b binding. Fab fragments of two additional antibodies recognizing cryptic sites (X49 and X50) and of two antibodies

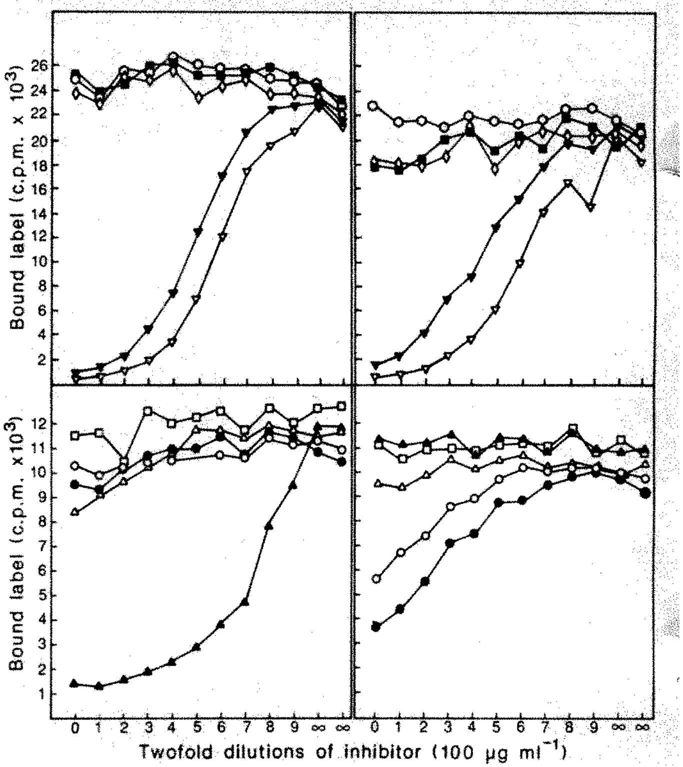

Fig. 2 Inhibition of ^{125}I-labelled LC_a (left panels) and ^{125}I-labelled LC_b (right panels) binding to clathrin heavy chain with Fab fragments of anti-light-chain antibodies. Iodinated LC_a and LC_b light chains were tested for binding to clathrin heavy chain in the presence of increasing dilutions of Fab fragments produced from anti-light chain antibodies. The two left-hand panels show effects on LC_a binding and the right-hand panels show effects on LC_b binding, of each inhibitor, indicated as follows: ■, LCB.6 Fab; ⬡, CVC.7 Fab; ◇, X50 Fab; ▽, LC_b; ▼, LC_a; □, anti-Leu 10 Fab; △, X49 Fab; ○, X45 Fab; ●, X43 Fab; ▲, X16 Fab. Unlabelled LC_a and LC_b each inhibit binding of both light chains[2,3]. Results shown here represent light-chain binding to heavy chain prepared by mild proteolysis of triskelions. Similar results were obtained when heavy chain was prepared by proteolysis of clathrin baskets.

Methods. Iodinated LC_a and LC_b were each tested for binding to clathrin heavy chain in the presence of dilutions of Fab fragments of anti-light chain antibodies or purified LC_a and LC_b, starting at 100 µg ml^{-1}, in a competitive solid-phase RIA[9]. Bovine brain LC_a and LC_b (purified and separated as in Fig. 1) were iodinated with 1 mCi ^{125}I-sodium iodide per 25 µg using Enzymobeads (Biorad). 5×10^5 c.p.m. LC_a or 2.5×10^5 c.p.m. LC_b in 20 µl phosphate-buffered saline (PBS), *p*H 7.4 with 0.5% bovine serum albumin (PBS-BSA) were used per assay. Clathrin heavy chains were prepared by elastase digestion of purified triskelions[11] at a 1:12 ratio of enzyme to protein. Heavy chains were applied to polyvinyl chloride plates at 350 µg ml^{-1} in 0.5 M Tris-HCl, 1 mM EDTA, *p*H 7.0 and the assay carried out in PBS-BSA. When basket-derived heavy chains were used, the assay was carried out under conditions maintaining basket stability[10]. Fab fragments were generated from monoclonal antibodies using established methods[20].

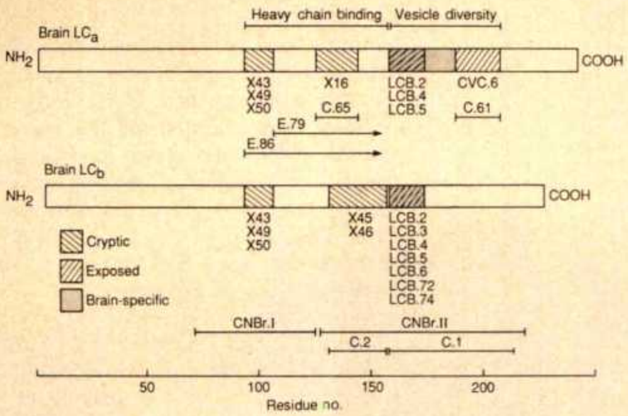

Fig. 3 Structural regions of the clathrin light chains mediating heavy-chain binding and coated vesicle diversity. The location of antibody-binding sites and light-chain peptides in the primary sequences of brain LC$_a$ and LC$_b$ are drawn to scale based on the data in ref. 6. Stipple, brain-specific insertion sequences. The LC$_a$ insertion sequence has 12 additional amino acids compared with the LC$_b$ insertion sequence. Antibody-binding sites were deduced by reactivity with peptides, and the reactive antibody is listed below the sequence containing its binding site (shaded region). Differential shading, sequences which contain exposed or cryptic antibody-binding sites. The protein sequences of peptides produced from clathrin light chains were determined[6], and the sequence spanned by each peptide is indicated below the map of each light chain from which it was derived.

Methods. Light-chain peptides were produced by digestion with clostripain (C peptides) or elastase (E peptides) or by CNBr cleavage, and fractionated or purified by reverse-phase HPLC[6]. The C.1 peptide extends from either residue 158 or 162 to 288, but is shown as starting with 158. The C.2 peptide extends from 131 to 157, based on relative molecular mass. It was sequenced up to residue 146. The carboxy-terminal amino acids of peptides E.79 and E.86 are not known but the CVC.6 site is included in E.86 but not in E.79. Antibody binding to peptides was measured by indirect solid-phase radioimmunoassay[9]. Aliquots of HPLC fractions containing purified peptides (3 μl at $\sim 100\ \mu g\ ml^{-1}$ in 0.1% trifluoroacetic acid) were dried onto wells of polyvinyl chloride plates. After rehydration in PBS, wells were saturated with PBS-BSA, then tested for binding of anti-light chain monoclonal antibodies (50 μl at 20 $\mu g\ ml^{-1}$ PBS-BSA or 50 μl hybridoma culture supernatant). Antibody binding was detected with ^{125}I-RAM. Each antibody reacted only with the peptides which are shown as spanning the antibody-binding site on each light chain, demonstrating no cross-contamination of peptides. Peptide-binding reactions were >10,000 c.p.m., with backgrounds of <1000 c.p.m.

binding to exposed sites (CVC.7 and LCB.6) had no effect on light chain–heavy chain reassociation. These results show that the cryptic antigenic sites recognized by X16, X45 and X43 are light-chain determinants involved in contacting the clathrin heavy chain, in both the triskelion and coated vesicle structures.

To identify light-chain sequences recognized by the monoclonal antibodies and thus to localize the exposed and cryptic regions, anti-light-chain antibodies were tested for binding to the purified peptides used for primary structure determination[6]. Fourteen of 16 antibodies tested reacted with isolated peptides, as measured by indirect radioimmunoassay (Fig. 3). The binding sites of the X16, CVC.6 and X45/X46 antibodies were determined by reactivity with single peptides C.65, C.61 and C.2, respectively. The X43/X49/X50 binding site was mapped by reactivity with the CNBr.I peptide from LC$_b$ and by differential binding to the E.86 and E.79 peptides which both contain the X16 site of LC$_a$. The brain-specific antibodies were mapped to the brain LC$_b$ insertion sequence, and the homogolous region of the brain LC$_a$ insertion sequence, based on binding to the C.1 peptide of LC$_b$.

These results define two distinct structural regions of the light chains. The exposed sites on LC$_a$ and LC$_b$ are located in residues 158–208, a region which contains the brain-specific insertion sequences. The cryptic determinants are localized to a region involving residues 93–157. All the antigenic determinants mapped are found in the hydrophilic sequences which span the carboxy-terminal 60% of each light chain and are characterized by their content of basic amino acids. It is possible that this property accounts for the greater apparent antigenicity of this region. Further examination of hydrophilicity plots of brain light-chain sequences indicates that reduced hydrophilicity at the beginning of the insertion sequences bisects the hydrophilic region in brain LC$_a$ and LC$_b$ (see Fig. 3 of ref. 6). This division corresponds exactly to the division between the cryptic and exposed sites detected by the anti-light-chain antibodies.

Localization of two structurally distinct regions of the clathrin light chains has revealed how different sequences could mediate separate functions. The first region (residues 93–157) is involved in binding the clathrin heavy chain. The sequence is predicted to be α-helical and includes the region of strongest homology (residues 124–149) with intermediate filament proteins[6]. This suggests that light chain–heavy chain interactions may involve contacts between a light-chain α-helix and a helix-binding structure on the heavy chain.

The second region (residues 158–208) is exposed to the cytoplasm and contains the brain-specific insertion sequences and the adjacent sequence recognized by the CVC.6 antibody (residues 188–208) on brain and non-brain LC$_a$. It has been previously shown that the CVC.6 antibody binds close to the triskelion vertex[11], but antibodies against other light chain determinants map further along the proximal part of the arm[2]. These data suggest that the light chain is oriented with its carboxy terminus toward the centre of the triskelion. In addition, this result localizes the CVC.6 binding site and the adjacent brain-specific sequence to a protruding point on the assembled coated vesicle[12]. The exposed location of the polymorphic light-chain sequences indicates a role in influencing diversity of coated vesicle function. This influence may be due to light-chain effects on the conformation of the triskelion vertex, resulting in diversity of coated vesicle size and shape[2,13]. Accessibility of polymorphic sequences also provides a potential recognition site for coated-vesicle binding proteins which could mediate tissue-specific functions.

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Old inflation and anisotropy

BARROW and Turner[1] have studied the effects of anisotropy on Guth's original model of inflation[2]. They concluded that anisotropy, if large, could prevent inflation from occurring. Though Guth's 'old inflation' suffers from the fatal 'graceful exit' problem (models which undergo sufficient inflation never again become radiation-dominated[3]), and has been supplanted by Linde's and Albrecht and Steinhardt's 'new inflation'[4,5], the work of Barrow and Turner is still frequently referred to. Further, it has been argued that their conclusions are incorrect, although we find the arguments of these authors to be invalid or inapplicable. For these reasons, we reexamined the paper by Barrow and Turner. In doing so, we have uncovered an error in their paper and find their basic conclusion to be wrong; large amounts of initial anisotropy do not, in general, prevent old inflation.

Guth's original model of inflation involves a first-order phase transition associated with spontaneous symmetry breaking (SSB) of a grand unified theory (GUT). SSB is affected when the scalar field ϕ acquires a vacuum expectation value, $\phi = \sigma$. At high temperatures ($T \gg T_c$, where T_c is the critical temperature for the phase transition) the finite temperature effective potential, $U_T(\phi)$, is minimized for $\phi = 0$, while at low temperatures ($T \leq T_c$) $U_T(\phi)$ achieves its minimum at $\phi = \sigma$, which signals SSB. For a first-order phase transition the symmetric state ($\phi = 0$) is still a local minimum of U_T for $T < T_c$, and is metastable for $T \leq T_c$ due to the potential barrier between $\phi = 0$ and $\phi = \sigma$. Because of this the Universe can exist in the metastable, symmetric state for $T \leq T_c$. While it does there is an enormous vacuum energy associated with the metastable (or false vacuum state), $U(\phi = 0) = 0(T_c^4)$ (where for $T \ll T_c$, $U_T \equiv U$ is temperature independent), and this vacuum energy drives an exponential expansion of the Universe (inflation). Because of the potential barrier between the false and true vacuum states, transition to the true vacuum must occur by the nucleation of bubbles of the true vacuum state which then expand at the speed of light[10].

In the context of the Friedmann-Robertson-Walker cosmology Guth has calculated the probability that a given point in space remains in the symmetric phase and finds that it is $e^{-F(t)}$ where

$$F(t) = \int \lambda(t_1) R^3(t_1) V(t, t_1)\, dt_1$$

$$V(t, t_1) = \frac{4\pi}{3}\left[\int_{t_1}^{t} \frac{d\theta}{R(\theta)}\right]^3$$

$R(t)$ is the Friedmann-Robertson-Walker scale factor, and $\lambda(t)$ the nucleation rate, taken to be constant once the phase transition has begun:

$$\lambda = \begin{cases} 0, & T > T_c \\ \lambda_0, & T \leq T_c \end{cases}.$$

[Throughout we use units where $\hbar = k = c = 1$ and $G \equiv m_{\rm pl}^{-2}$.] Inflation begins when $T \simeq T_c$, at $t \equiv t_{\rm GUT} \simeq 1/3\chi$, where $\chi^2 = 8\pi U(0)/3m_{\rm pl}^2 = O(T_c^4/m_{\rm pl}^2)$. During inflation, $R \sim e^{\chi t}$, and

$$F(t) = \frac{4\pi\lambda_0 t}{3\chi^3}$$

When $F(t)$ becomes of order unity, most of space is in the true vacuum phase, and the inflationary phase is over. A minimum of about 60 e-folds of inflation is required in order to solve the horizon and flatness problems[2]. Since inflation ends at $t = t_*$ where $F(t_*) = 1$, this implies that for inflation to be successful the nucleation rate λ_0 must be $< 3\chi^4/4\pi N \simeq \chi^4/80\pi$ where the number of e-folds of inflation, N, is taken to be ≥ 60.

Consider the effect of initial anisotropy on inflation. Following Barrow and Turner, we assume that at early times, the energy density of the Universe is dominated by 'anisotropy energy density', Σ^2/R^6, and that the analogue Friedmann equation for the mean scale factor $R(t)$ (valid for all Bianchi I models) is

$$\left(\frac{\dot{R}}{R}\right)^2 = \chi^2 + \frac{\Sigma^2}{R^6} + \frac{8\pi}{3}\frac{T^4}{m_{\rm pl}^2}$$

where $\Sigma^2/R^6 \gg T^4/m_{\rm pl}^2$, χ^2 represents the contribution of the vacuum energy, and the temperature $T \propto R^{-1}$. (This equation is only valid if the Universe is still in the symmetric state, for $t \leq t_*$.) At early times ($t \leq t_V = 1/3\chi$) anisotropy dominates and $R \sim t^{1/3}$, while at late times ($t \geq t_V = 1/3\chi$) vacuum energy dominates and $R \sim e^{\chi t}$. Ignoring the subdominant T^4 term, evolution of the mean scale factor $R(t)$ is given by

$$R^3 = \frac{\Sigma}{\chi} \sinh 3\chi t$$

Note that vacuum energy begins to dominate at $t \simeq 1/3\chi$, independent of the level of anisotropy. This fact, or equivalently, that Σ^2/R^6 is independent of Σ, has been used[6-9] to argue that anisotropy has no effect on the phase transition or inflationary process. This misses an essential point, the level of anisotropy does influence $t_{\rm GUT}$, the time at which $T \simeq T_c$ and bubble nucleation can commence. In the presence of anisotropy

$$t_{\rm GUT} = \gamma^{-1/2}/3\chi = \gamma^{-1/2} t_V$$

where $\gamma \equiv (\Sigma^2/R^6/\chi^2)|_{T = T_c}$, that is, γ is the ratio of the anisotropy energy density to that in radiation when $T \simeq T_c$. The effect of larger anisotropy then, is to push $t_{\rm GUT}$ to earlier times, so that bubble nucleation can commence sooner.

During the anisotropy-dominated phase, $R \sim t^{1/3}$ and $F(t) = 9\pi\lambda_0 t^4/80$, which implies that $t_* \simeq (80/9\pi\lambda_0)^{1/4}$. If $\lambda_0 > 720\chi^4/\pi$, then $t_* < t_V$, and the transition to the true vacuum state will be complete before the vacuum energy comes to dominate, thereby preventing inflation. But if this inequality is satisfied, the previous constraint on nucleation rate implies that sufficient inflation would not have occurred in the absence of anisotropy either. That is, anisotropy does not spoil inflation that would have otherwise been successful. It is also known that initial anisotropy does not adversely affect new inflation[11,12]. As Barrow and Turner pointed out and others have since studied in more detail[11,12], more than 60 or so e-folds of inflation will damp any initial anisotropy, regardless of amplitude, to an undetectable level today. Old inflation then also solves the anisotropy problem.

The error made by Barrow and Turner occurred just below equations (5), (6) where they incorrectly interchanged $t_{\rm GUT}$ and t_V. In a radiation-dominated Friedmann Universe, both t_V and $t_{\rm GUT}$ are $O(m_{\rm pl}/T_c^2)$, and t_V is independent of the level of anisotropy. However, as discussed above, in the presence of anisotropy $t_{\rm GUT} \simeq \gamma^{-1/2} t_V$. This led Barrow and Turner to conclude that by raising the initial level of anisotropy one could always make the Universe anisotropy-dominated at $t = t_*$, thereby prevent inflation. Let us be more specific. While the Universe is anisotropy-dominated the ratio of anisotropy energy density to vacuum energy density decreases as t^{-2}, so at $t = t_*$

$$[(\Sigma^2/R^6)/\chi^2]|_{t=t_*} = \gamma(t_{\rm GUT}/t_*)^2$$
$$= (\pi\lambda_0/720)^{1/2}/\chi^2$$

which is independent of γ and only greater than unity if $\lambda_0 > 720\chi^4/\pi$, the same condition found previously. By mistakenly using t_V for $t_{\rm GUT}$ in this equation Barrow and Turner found that the ratio of anisotropy to vacuum energy density at $t = t_*$ to be γ times the correct expression, and concluded that sufficient initial anisotropy could guarantee that the Universe was anisotropy-dominated at $t = t_*$.

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Reader Service No.22

Beating the big battalions

Publishers are not serving some of their authors or student customers as well as they might. Wallace S. Broecker describes his experience of desk-top publishing, and suggests how authors could make themselves a force to contend with.

IN MY subject, geochemistry, there is a dearth of textbooks designed for use in advanced undergraduate and first-level graduate courses; further, those textbooks we do have are often not written by the most appropriate people. Very often we are forced to teach entirely from articles published in journals, or from books with a large number of contributors, which is an inefficient and uninspiring means of presenting the subject. I suspect that the situation is much the same in many of the more specialized but rapidly growing fields of science and engineering.

There are several reasons for this state of affairs. One of them, I contend, is that the publishing industry has a number of practices which discourage creative authors. I list a few.

(1) More daring and inventive proposals are rejected because of the unwillingness of publishers to take risks. Their tendency is to follow the format for well-defined courses run at many universities; the result is books that are alike in content and design. This is being competitive but unimaginative.

(2) Once the manuscript is submitted, the publisher is inclined to view it as his property and dominates decisions about format, cover design, marketing and so on. The author's personality is removed by editing out whimsy and anything that seems unconventional.

(3) Publishers provide a professional service of copy-editing, production, marketing and distribution, but at the cost of supporting large overheads. They are also commercial organizations, interested above all in making a profit. The results are that books are expensive, especially those for the less well-populated advanced courses, and that authors rarely see a decent financial return for their efforts.

Is there an alternative? I have conducted two self-publishing experiments which demonstrate to me that there is. The first book, entitled *Tracers in the Sea*, is 700 pages long and is a treatise on dissolved chemical species in the sea. Since its publication in 1982 it has sold 3,000 copies. All the costs have been recovered; the account is now in the black. The second, *How to Build a Habitable Planet* (280pp.), is aimed at university students and non-scientists interested in the physical heritage of the Earth. Like the previous book it is distributed by Lamont–Doherty Geological Observatory, but because it was published in December 1986 as yet we have little indication of how well it will sell.

As never before, the combination of word-processor, computer layout and laser printing brings within every author's grasp the possibility of producing his or her own book. As those of us who use the 'author-ready copy' option for journal articles already know, this technology is in place. We have only to learn how to use it. The manuscript is typed and edited on a word-processor. The disk from the word-processor (including figure captions and tables) is transferred to a computer system, on which the page layouts are done and which also scans the line-drawings. A laser printer is then used to make camera-ready copy for the printer, who needs only to drop any photographs into position.

One might ask, "Isn't it painful to handle all the production details?". To me it was a pleasure after all the sweat I had put into the writing and revisions. The time required was surely less than 5 per cent of the total. Much of this went into proofreading and other tasks with which all authors have to contend. Above all, the book remained mine.

Then, where's the rub? Why doesn't everybody do it? There are two big problems. One has to do with financial backing. In my case there was the matter of $30,000 to be found (see the table on production costs). I borrowed the money from a combination of sources. As can be seen, the total production cost, including all the typing (I write by hand, in pencil, by rivers, in cafeterias . . .) and drafting, came to just under $6 per book printed. To the extent that an author did his or her own drafting, typing and computer layout, the costs could be reduced significantly. We estimate the distribution costs to be about $5 per book. At the discount price of $18 for the softcover edition and $30 for the hardcover, I must sell a mix of about 2,200 books before the break-even point is reached (see the table on financial outlook).

Summary of production costs for
How to Build a Habitable Planet

Typing and drafting (in house)	$13,000
Photographic fees	1,000
Copy editing (freelance)	1,000
Computer layout (commercial)	3,000
Printing (5,500 copies)	12,000
Total	$30,000

Financial outlook

First printing (5,500 copies)		
Production costs	–$	30,000
Distribution costs	–	25,000
Sales receipts	+	117,000
'Profit'	$	62,000
Second printing (5,500 copies)		
Production costs	–$	12,000
Distribution costs	–	25,000
Sales receipts	+	117,000
'Profit'	$	80,000

This calculation assumes that all the books will be sold, which of course they might not be. Herein lies the risk. However, most reputable textbooks should easily exceed the break-even sales point (for this book about 2,200 copies).

The other problem has to do with marketing and distribution. Herein lies the real pain. My first book was aimed at a sufficiently narrow audience and was unusual enough that a combination of help from friends, careful advertising and word of mouth did the job. The costs were repaid and I received a modest royalty. Further, at $35 the book sold for less than one-half the price a publisher would have charged. This brought it into a range where students could purchase it out of interest and not only out of necessity. For my second book, the task will be more difficult. As there is no ready-made audience, I will have to work hard to create one.

Thus, although technology has removed the production problem, the problems associated with financing and distribution will force most authors to put themselves in the hands of a publisher. Part of the answer may lie in distribution co-operatives which cover a broad enough part of a given field to become viable. Participants would produce their own books (with advice from the co-op) and then use advertising and distribution services of the co-op. The other part of the answer may lie in grants from charitable foundations to provide the money required for typing, drafting, layout and printing.

I am convinced that one important step towards remedying the lack of quality textbooks in certain areas of science is to force the publishing industry to think again about the way it treats its authors and serves its student customers. This can be done through competition created by the alternative publishing route outlined above. The production technology is already accessible to most authors. What remains to be developed are mechanisms to permit aspiring authors to obtain the funds needed for the production of their books, and co-operative systems by which those books could be marketed and distributed. □

Wallace S. Broecker is at the Lamont–Doherty Geological Observatory of Columbia University, Palisades, New York 10964, USA.

Varieties of quantum lore

R. B. Jones

Quantum Mechanics in Simple Matrix Form. By Thomas F. Jordan. *Wiley: 1986. Pp.257. Pbk $26.50, £25.70.*
Quantum Mechanics. By Ta-You Wu. *World Scientific: 1986. Pp. 417. £33.15, $41.40.*
Non-Relativistic Quantum Mechanics. By Anton Z. Capri. *Benjamin/Cummings: 1986. Pp. 653. $34.95, £36.95.*
Quantum Mechanics and the Particles of Nature: An Outline for Mathematicians. By A. Sudbery. *Cambridge University Press: 1986. Pp.358. Hbk £40, $60; pbk £15, $25.*
Quantum Mechanics, 2nd Edn. By A. I. M. Rae. *Adam Hilger: 1986. Pp. 247. £9.95, $22.*

There are nine and sixty ways of constructing tribal lays, and every single one of them is right.

KIPLING was unaware of the tribe of quantum mechanics, but he unerringly described their prolixity in composing accounts of their arcane lore. Of the five well-produced and useful volumes reviewed here only two are straightforward textbooks, the other three having features or aims that are as surprising as they are interesting.

Quantum Mechanics in Simple Matrix Form is a charming book written for "gifted high-school students" and for "most people who read the *Scientific American*". In his first 16 chapters Jordan succeeds admirably in reaching this audience by using the simplest quantum system, the spinning electron, to illustrate the assumptions of quantum mechanics, to explore the ideas of Bohr and Einstein, and finally to explain how experimental tests of the ideas of John Bell have shown convincingly that quantum mechanics does not describe an objectively existing local reality in nature.

In the second half of the book the going gets rougher for the non-mathematical reader, as the use of matrix methods is enlarged to treat angular momentum, the hydrogen atom, and symmetry operations of rotation and translation. This, then, is not a conventional text. Quite apart from all those high-school students, however, it should be read by undergraduates doing technical courses in quantum mechanics and by professional quantum physicists who will find it a refreshing presentation of simple and profound ideas.

Ta-You Wu learned quantum mechanics with Goudsmit and attended the lectures of Heisenberg and Uhlenbeck; later, as a teacher in wartime China, he taught the subject to C.N. Yang and T.D. Lee. The latter luminaries have contributed forewords to their old teacher's fascinating textbook, in which he explains the subject by meticulously tracing its historical roots.

Familiar himself with all the details of the old quantum theory of pre-1925, Professor Wu leads the student through the astonishing successes of this period and then draws on an encyclopaedic knowledge of the literature of 1925–1928 to teach the subject as it was discovered then. Separate chapters trace the miraculous and almost simultaneous discoveries of matrix mechanics and wave mechanics. All the basic models and techniques of a modern course are covered clearly, with particularly detailed presentation of the special functions used for the oscillator, angular momentum and the hydrogen atom. There are good accounts of perturbation theory, and a brief mention of scattering problems and concepts of the Green function.

In the last three chapters, Professor Wu draws on his specialist knowledge of atomic and molecular spectra to describe approximation methods for energy levels of many electron systems; included here is an extended treatment of how different angular momentum coupling schemes play a role across the periodic table. This book is probably too detailed, and in places too advanced, for a one-semester first undergraduate course. But it is highly recommended as supplementary reading for advanced undergraduates or for first-year graduate students.

Anton Capri's book is an extensive and modern treatment of nonrelativistic quantum mechanics suitable for a two-semester undergraduate or one-semester graduate course. Essentially all of the standard topics are covered, although with an unstated bias towards the physics of nuclei and elementary particles as compared with condensed matter physics.

The author makes a noteworthy attempt to introduce more mathematical detail than is usual in his discussion of the linear space structure of quantum mechanics. The ideas of Schwartz spaces, dual spaces and rigged Hilbert spaces, and the distinction between strong and weak convergence, are clearly introduced, and the treatment of standard problems and techniques is uniformly good. I was pleased by the discussion of the WKB method and of the Glauber approximation for scattering theory. Capri is aware of the quantum Hall effect, and includes a nice account of the effect of a magnetic field and gauge invariance upon electron motion. He concludes with an introduction to second quantization and a brief description of density matrix methods. This book should be carefully considered for use in advanced undergraduate or early graduate courses.

Anthony Sudbery's *Quantum Mechanics and the Particles of Nature* is written for mathematicians who want to learn about elementary particle physics. After an introductory look at the particle jungle, with its shy inhabitants the quarks and gluons, there is a three-chapter exposition of ordinary nonrelativistic quantum mechanics; here the reader is expected to understand quickly how rigged Hilbert spaces, tensor product spaces and Lie groups play a role in the theory.

Then comes a glorious chapter on quantum metaphysics, in which no less than nine (not exhaustive) interpretations of the basic quantum postulates and of the idea of measurement are explored and compared. In spite of the fact that, as Richard Feynman says, "Nobody knows how it can be like that", these metaphysical problems continue to exercise physicists and philosophers and are well surveyed here. In the next chapter the particle jungle is mapped out in detail, from isospin to flavour spectroscopy, from colour to asymptotic freedom, from the Salam–Weinberg theory to grand unification and supersymmetry. Sudbery's description is generally sound, except that he seems to believe that the top quark has been definitively established whereas the experimental group who are alleged to have found it are much more modest in their claims. The last chapter introduces relativistic quantum gauge theory, including hidden symmetry and the Higgs mechanism.

This volume is not to be regarded as a textbook. Rather, it is a stimulating monograph on how we attempt to grasp intellectually the essence of the elementary building blocks of the Universe.

The second edition of Alastair Rae's *Quantum Mechanics* does not differ greatly from its predecessor, reviewed here in 1983. The changes include a more careful treatment of spin–orbit interactions and the Zeeman effect, elaboration of the discussion of degenerate perturbation theory and an improved account of the uncertainty principle. A set of detailed hints to help with the exercises has been incorporated as well.

Of the five books reviewed, this is the one best suited to a straightforward one-semester introductory undergraduate course. It contains much of the quantum material required for an Honours BSc physics course, and lacks only consideration of some topics of relevance to condensed matter physics such as the role of the electromagnetic vector potential in quantum mechanics and the effect of spatially periodic potentials. □

R.B. Jones is a Lecturer in the Department of Physics, Queen Mary College, Mile End Road, London E1 4NS, UK.

Central concerns in physics

James Lowe

An Introduction to Nuclear Physics. By W.N. Cottingham and D.A. Greenwood. *Cambridge University Press: 1986. Pp. 210. Hbk £25, $44.50; pbk £8.95, $14.95.*
Nuclear Physics: Energy and Matter. By J.M. Pearson. *Adam Hilger: 1986. Pp. 250. £20, $44.*

BOTH of these books are intended as introductory, undergraduate texts for courses in modern nuclear physics. Similar criteria have guided the choice of material in each case, but the end results are rather different.

In their book, Cottingham and Greenwood present an interesting selection of topics. The rationale behind the choice of them, as stated by the authors, is that as well as learning about the basic properties of the nucleus readers should acquire enough information to enable them to understand the problems of present-day nuclear technology and to make informed judgements on them. Thus, for example, compound-nucleus reactions, which are very relevant to nuclear power generation, are discussed in detail, while direct reactions receive much less attention. Similarly, the restriction of the account of excited states to those below 10 MeV precludes consideration of such topics as giant resonances, which have no practical application but have surely been important in the development of our understanding of the nucleus. By contrast, nuclear reactors are treated in some detail.

Nevertheless, this is in no way an applied physics textbook. The authors invariably make the basic physics clear, and particularly helpful is the brief description of the properties of elementary particles in the opening chapters, which puts the remainder of the book into proper context. The sections on nuclear astrophysics are also very welcome; the treatment both of stellar formation and of nucleosynthesis brings out clearly the basic physical principles of the subject. With the one reservation over the omission of certain topics, I can warmly recommend this volume. In the preface, the authors acknowledge their debt to their teacher, Sir Rudolf Peierls; his influence is, I think, often discernible in the book.

Pearson's text is also slanted towards applications, but here the emphasis is much stronger. The author specifically avoids writing for the specialist student, and instead provides a background for what he calls the "large-scale manifestations" of nuclear physics, such as nuclear power generation and nuclear applications in medicine. It follows that his discussion is often more concerned with conveying facts than with emphasizing physical principles or the relation of a topic to the rest of physics. Occasionally, I feel that this is taken too far. For example, the account of gyromagnetic ratios of particles refers to the 'classical' value of unity. The experimental values for the proton, neutron and electron are then quoted without any real comment on their interpretation or on the significance of the closeness of the electron gyromagnetic ratio to 2.

Not all aspects are treated in this way, however: indeed, to give one example, the discussion of the independent particle model deals with the basic quantum mechanics of the problem better than do many other books. Nor is the coverage of applied nuclear physics limited to man-made applications; a significant fraction of the book is devoted to nuclear astrophysics.

These two books, then, declare a common aim and are remarkably similar in the material they contain. If buyers have to choose between them, they must ask why they are studying nuclear physics. If it is primarily for the applications, in the nuclear industry or other areas, then Pearson's book will probably be the more useful. If it is to understand the basic principles and to appreciate the relation of nuclear physics to other branches of physics, while seeking some general education in the implications of the subject for everyday life, then the choice is likely to be Cottingham and Greenwood. ☐

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- Cambridge University Press has reissued in paperback Arthur Eddington's classic *Space, Time and Gravitation: An Outline of the General Relativity Theory,* which first appeared in 1920. The new edition includes a foreword by Hermann Bondi; price is £8.95, $14.95.

Brazilian plasmas

Alan Phelps

Fundamentals of Plasma Physics. By J. A. Bittencourt. *Pergamon:1986. Pp.711. Hbk £55, $83; pbk £22, $32.*

HITHERTO, textbooks on plasma physics have originated from authors in the United States, Europe, the Soviet Union or Japan, thus reflecting the main centres of research activity in the subject. The developing efforts in plasma physics elsewhere in the world will result in the appearance of texts such as this one from Brazil. The book is written for advanced undergraduate and first-year graduate students, and is also designed to be suitable for self-learning. The style and idiom have a North American flavour.

Until recently there was only a small number of plasma textbooks which were both appropriate for undergraduate use and which used SI units or their equivalent. Bittencourt's book is pitched at the right level for its intended readers, uses rationalized MKSA units and includes problems at the end of each chapter, and it should therefore be of wide appeal. The usefulness of the problems for both teaching and self-learning would have been enhanced if solutions had been included, though in partial compensation the problems are phrased in a helpful manner which often leads to the answer. More material is included than can be covered normally in one semester and the user therefore needs to be selective. This is not a disadvantage, but lecturers trying to give a balanced introductory course may find that the lack of description of experimental aspects and of the contemporary methods of plasma diagnostics means that they have to draw upon additional sources of information.

The title is very appropriate, as the author does indeed address the fundamentals. Although the mathematics required is not above undergraduate level, the sheer number of equations embedded in the text implies that the book will be enjoyed more by theoretically inclined students than by experimentalists. Those intending to pursue research into space plasma physics should find the coverage is relevant to their needs.

There are some drawbacks. The exposition is clear, logical and academically satisfying, but at the price of conveying only in part the sense of excitement that characterizes plasma physics at present and that is so important in motivating the average student. No distinction is made between the cyclotron frequency and the traditionally defined Larmor frequency. Also, in grouping the main experimental efforts for achieving fusion — (1) open systems (2) closed systems (3) theta pinch devices (4) laser-pellet fusion — the author over-emphasizes the current importance to fusion of the theta pinch devices, while 'inertially-confined plasmas' would have been a better description for (4) and would then have included other drivers such as particle beams, as well as lasers. There are, too, a number of typographical errors, but no more than one would expect in a book of this length.

These are minor points. *Fundamentals of Plasma Physics* should be acquired by most science libraries, though I expect students will use it mainly to supplement their other textbooks on the subject.

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Unearthly exercises

David W. Hughes

Astronomical Methods and Calculations. By Agnes Acker and Carlos Jaschek. *Wiley: 1986. Pp.343. Hbk $34.95, £38.50; pbk $26.35, £14.95.*

SCIENCE is quantitative and astronomy is no exception. But astronomy has a special piquancy because the problem of measuring such fundamental parameters as mass, size, temperature and composition is all the more demanding when the objects of interest are so far away.

Take as an example the calculation of the distance between our Galaxy and its near neighbour Andromeda (Messier 31). In 1919 Lundmark used novae observations to obtain a value of 200 kpc (1 kpc ≡ 3×10^{16} km). Bright, non-variable stars indicated that it should only be 100 kpc. By 1922 Öpik had obtained a value of 450 kpc, and Lundmark's recalculation in 1925 gave 430 kpc. In the same year, using Cepheids, Hubble plumped for 275 kpc. Returning to the problem in 1948 Lundmark measured the distance to be 405 kpc and Baade in 1952 found a value of 500 kpc. The consensus today is that Andromeda is about 660 kpc away. This variation carries through to our estimations of energy output and has an effect on such basic cosmological quantities as the density of the Universe.

All astronomers need to understand the methods used to measure fundamental quantities and must be able to assess the reliability of the values obtained. The normal way of developing this proficiency is by confronting students with raw data and then sitting over them in the laboratory or observatory until they have worked through to an end result. Laboratory supervisors have to rely on a range of texts to help them devise meaningful exercises, and *Astronomical Methods and Calculations* is another to add to their book list (assuming, that is, that they do not already have in their possession the original French edition, which was published by Masson in 1981).

Acker and Jaschek provide the bare bones of 112 exercises, together (thank goodness) with a brief résumé of the expected solutions. There are four sections. The first concentrates on traditional astronomy, stellar co-ordinates, the determination of time, refraction and stellar motion. The second deals with the Sun and the planets, measuring distances using radar and the transits of Venus, assessing masses, sizes, shapes, temperatures, albedos and solar parameters such as limb darkening and curves of growth. The authors then turn to the stars and our Galaxy, and consider such topics as rotation, temperature scales, luminosity functions, Cepheid distribution and interstellar extinction. The final section deals with galaxies, their shapes and spectra, spin, distances, masses, clustering and velocities.

The reader is presupposed to have a considerable knowledge of astronomy, physics and mathematics, and most of the exercises will need amplification before they can be put before the average university student. There is a fountain of good ideas here, but one in which references are few and cross-referencing is far too meagre. And accuracy remains a problem. Little help is given to enable readers to differentiate between systematic and experimental error, so they will still have difficulty, for example, in finding out how far away Andromeda really is. ☐

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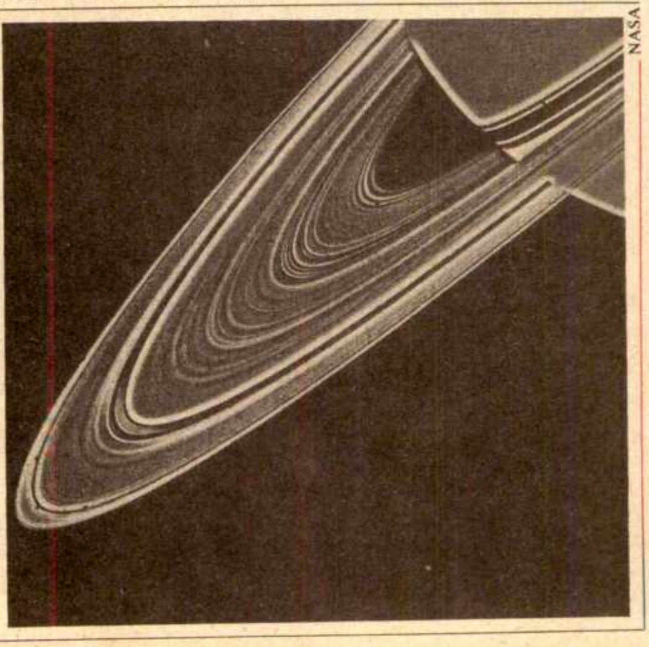

In a whirl — the rings of Saturn, viewed from Voyager 2 in August 1981. The picture is reproduced from a colour original in the fifth edition of The Physical Universe, *by Konrad B. Krauskopf and Arthur Beiser, published last year by McGraw-Hill.*

Making short work of heat

Tony Guénault

Elements of Thermodynamics. By Martin C. Martin. *Prentice-Hall: 1986. Pp.225. $39.33, £42.10.*

STUDENTS and teachers alike recognize the need for accessible textbooks, ones that cover a subject in not much more detail than the corresponding lecture course. Here we have a praiseworthy attempt to provide a text for a short course on thermodynamics for middle-year physics or engineering students. This is a worthwhile target area, although one in which there is competent opposition.

Martin's book has some strong points in its favour. The choice of topics is imaginative, and an introduction to the statistical approach is included. The level of the writing is well judged, although some readers might prefer fewer equations in places. And the production quality is very high, the work being well typeset and printed, making it a pleasure to pick up and read.

However, in spite of much to admire, this is not a book that inspires overall confidence. One problem is the basic approach to the subject. For example, it is surprising to discuss temperature before considering thermal equilibrium, and to see work introduced as something done by a fluid rather than against an externally applied (balancing) force. Throughout, more care and attention could have been given to the logical framework without affecting the level or length of the book. Thermodynamics should be an appealingly and remorselessly logical subject.

Given my own interests, and particularly because a picture of the fountain effect appears on the front cover, I turned quickly to the chapter on low-temperature physics. Here, one of the biggest disappointments is the illustrations. Several graphs look like artists' impressions of other artists' impressions, rather than depictions of the real thing. The phase diagram of helium-4 has a strange look to it, but that of the (lambda?) heat capacity is positively grotesque. And mention of the "more accurate" lambda point of 2.189K makes one uneasy — a value around 2.17K has been accepted for some while now. Elsewhere there are other similarly unrealistic pictures (for instance of black-body radiation and of critical isotherms). These and other small unsatisfactory details rather spoil the impact of what is in some ways an attractive textbook. ☐

Tony Guénault is a Reader in the Department of Physics, University of Lancaster, Lancaster LA1 4YB, UK.

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Model exposition

Jean Palutikof

Contemporary Climatology. By Ann Henderson-Sellers and Peter J. Robinson. *Longman/Wiley: 1986. Pp.439. Pbk £12.95, $21.95.*

Is THERE really room for another undergraduate textbook in climatology? I opened *Contemporary Climatology* with a somewhat world-weary sense of *déjà vu*, but was pleasantly surprised by the presentation, content and style. The book covers the full range of topics to be expected from a basic text, with useful sections on instrumentation and on the applications of climate information to economic (particularly agricultural) planning. The writing and the illustrations are of a high standard, and they combine well to ensure the reader's understanding of the subject matter. In addition, there is a helpful glossary of terms.

The most successful section is undoubtedly that on climate models. This is a rapidly advancing and increasingly important area of climate and climate-change studies; global-scale models are used to investigate problems as diverse as the nuclear winter (or autumn, if you prefer) and the Sahelian drought, while on a local scale models are used in applied climatology to look, for example, at water resources and crop–climate relationships. Yet adequate treatment of modelling theory and practice, particularly the long-term requirements of climatologists rather than the short-term forecasting needs of meteorologists, is rare in undergraduate texts. Here we are presented with about 40 pages of lucid explanation and illustration which should become the standard student reference on the topic. The other particularly enjoyable and educational aspect is the use of satellite images to illustrate points made in the text. Indeed, throughout the book frequent reference is made to the relevance of data gained from space to climatic research.

Overall, it is in the treatment of the most recent (particularly high-technology) developments in climatology that this book succeeds best. Conversely the weakest section is that on the most traditional branch of the subject — climatic classifications. The most annoying general feature is the failure to reference sources of information in either the text or figure captions; to follow up a particular reference the reader has to hunt through the Acknowledgements, a daunting task that few would pursue. But these are small criticisms of a book that seems destined to be widely used as a course text. □

Jean Palutikof is a Senior Research Associate in the Climatic Research Unit, University of East Anglia, Norwich NR4 7TJ, UK.

Ordered elements of sea and air

Malcolm Walker

The Atmosphere and Ocean: A Physical Introduction. By Neil Wells. *Taylor & Francis: 1986. Pp.347. Hbk £29, $52; pbk £15, $27.*

ACCORDING to the publisher's blurb, *"The Atmosphere and Ocean* is unique in bringing together the diverse concepts and ideas of meteorologists, atmospheric physicists and oceanographers into a single coherent account of our fluid environment, placing the emphasis on the physical ideas and mechanisms rather than on the mathematics". As co-author of a book with essentially the same theme *(The Ocean–Atmosphere System,* published by Longman in 1977), I was a trifle vexed by this claim; moreover, the designs on the front covers of the two books are similar, both adopting the waterspout as a motif to represent the coupling between ocean and atmosphere.

But these are minor complaints, not least because the styles of the books are very different. *The Ocean–Atmosphere System* largely takes the form of a review, whereas *The Atmosphere and Ocean* is an undergraduate textbook, concerned mainly with physical processes and demanding of its readers a greater competence in mathematics and physics. Indeed, without a subsidiary course in mathematics to introduce concepts not normally encountered at school, undergraduates will surely struggle to follow some sections. The author no doubt assumed that such a course would be provided for students using his book formally.

On the whole, however, *The Atmosphere and Ocean* is an excellent supporting text for undergraduate courses in marine science. There is little to criticize: one or two symbols are not defined and a few explanations are perhaps over-concise. Apart from the occasional lapse of proof reading, that is all. The overall impression is one of a book over which a great deal of care has been taken, by both author and publisher.

For the most part, difficult concepts are explained clearly and the book is scientifically sound. Furthermore, the order of the contents has been carefully thought out. The ten chapters lead the reader logically from an account of the Solar System and solar radiation to a discussion of climatic variability and predictability, through studies of the composition and properties of the ocean and atmosphere, vertical temperature profiles, atmospheric and oceanic stability, water in the atmosphere, global budgets of heat, water and salt, ways of measuring winds and currents, the influence of Earth's rotation on fluid motion, the nature of waves and tides, and energy transfer in the ocean–atmosphere system.

In short, *The Atmosphere and Ocean* can be strongly recommended as a textbook for undergraduate courses in marine science. I expect it to be widely adopted as such. □

Malcolm Walker is a Senior Lecturer in the Department of Maritime Studies, UWIST, Cardiff CF1 3EU, UK.

Learning on a plate

J.R. Cann

Marine Geology: A Planet Earth Perspective. By Roger N. Anderson. *Wiley: 1986. Pp.328. Pbk $31.50, £21.80.*
Plate Tectonics: How It Works. By Allan Cox and Robert Brian Hart. *Blackwell Scientific: 1986. Pp.392. Pbk $29.95, £19.75.*

IT IS astonishingly difficult to find good textbooks for advanced undergraduate courses on plate tectonics. After all, the plate tectonic revolution has been more or less complete since September 1968, when the final touches of gilding were applied in a memorable issue of *Journal of Geophysical Research*. Yet the best sources for teaching are still the original papers themselves. "Come now, Joe", I hear all my plate tectonic friends out there saying, "that's exactly as it should be. Give the young people a chance to experience the excitement just as we felt it back in '63 or '65!". But is this really the best way to teach? Remember the story that McKenzie and Morgan very nearly did not bother to publish their paper on evolution of triple junctions because they felt it was all too obvious — and that paper is still pretty nearly impenetrable, even for a bright student. And many of the classic papers have in one sense or another been superseded by later work, which is not tidily collected together anywhere. So yes, I do think there is a place for undergraduate texts on plate tectonics and related matters, and it is interesting to welcome two here, both written by practitioners of no mean talent themselves.

Roger Anderson's is the more conventional. It is a general account of marine geology, in some ways a condensed and more accessible version of Kennett's excellent *Marine Geology* (Prentice-Hall, 1982), but with considerably greater emphasis on solid-Earth aspects such as the geophysics of plate tectonics, and the geology of mid-ocean ridges and subduction zones. It is interesting that where Kennett's book is weaker, Anderson's is stronger and vice versa. Thus in handling marine stratigraphy Anderson uses stock diagrams and makes subtle but definite errors in the handling of the biology and oceanography. On the other hand, his account of the subduction terrains of the western Pacific is entirely up to date, and illustrated with fine and striking graphics. It is unfortunate, though, that some of the diagrams in the book are reproductions in murky greys of colour originals, for which I fear the publisher must take responsibility. In addition to the more standard material of marine geology the book contains chapters on plate tectonics and continental geology, on the evolution of sedimentary basins and on mechanisms of plate tectonics. I shall be recommending this book to my students, and especially to those who are struggling with some of the more complex early papers.

Cox and Hart's book is very different. It is much more unconventional, taking a quantitative approach that is difficult to characterize in a word. Much of it deals with the geometry of plate tectonics, Eulerian poles, evolution of triple junctions (explaining McKenzie and Morgan very clearly), instantaneous rotations and finite rotations. But there are sections on earthquake focal mechanism solutions and on palaeomagnetism (not altogether surprisingly, considering the authors). Another way of seeing a unifying theme would be in terms of spherical geometry, which many of the topics in the book have in common. In some ways this theme is also the book's undoing, especially as finite rotations are gradually developed, and there are pages of detail that tend to obscure the flow of the argument. Another irritation is the folksy style of some of the linking prose — not that I'm against folksiness in itself, but when it is inaccurate (as in the fictionalization of a submersible dive) then I start to have doubts.

However this book is a most important addition to geological textbooks. It provides clear and systematic treatment of some of the most difficult and elusive aspects of plate tectonics, aspects which I suspect many people omit from their courses because of the problems of teaching them, but which are really fundamental to the subject. This is not a general text on plate tectonics, despite the title, but it is an excellent book that deserves to be widely used. ☐

J.R. Cann is a Professor in the Department of Geology, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, UK.

- *The Physics of the Earth's Core: An Introduction*, by Paul Melchior, is intended to provide a basis upon which astronomers, geodesists and others can build an understanding of the latest developments in the study of the Earth's interior. Publisher is Pergamon, price is hbk £26.75, $39.95; pbk £13.35, $19.95.

Resourceful science

Stephen E. Kesler

The Geology of Ore Deposits. By John M. Guilbert and Charles F. Park, Jr. *W. H. Freeman: 1986. Pp. 985. Hbk $47.95, £29.95; pbk £19.50.*

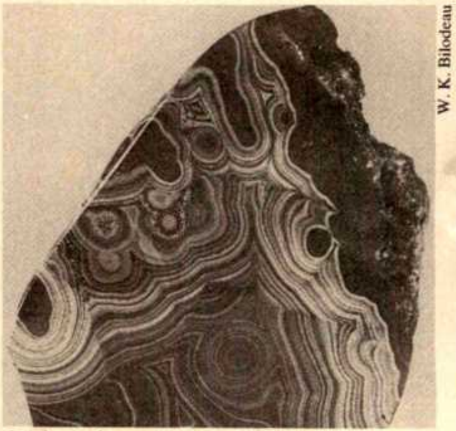
Ore Deposit Geology. By Richard Edwards and Keith Atkinson. *Chapman & Hall/Methuen: 1986. Pp. 466. Hbk £40, $75; pbk £19.50, $33.*

MOST geologists are descended from the early naturalists, whose interest in the Earth reflected purely intellectual curiosity. Not so the economic geologist, who traces his roots in part to the pragmatic prospector. Perhaps driven by a need to purge this non-scientific skeleton from our ancestral closet, modern economic geologists have emphasized the scientific aspects of the subject. Western students of economic geology rarely learn about the engineering and economic factors that make an ore deposit mineable, and they have little or no understanding of how to explore for new deposits. It is worthwhile contemplating whether this is partly responsible for the poor showing of many mineral companies during the lean years of the 1980s.

The two books under review reflect the schizophrenic heritage of economic geology. Guilbert and Park emphasize "the principles and data fundamental to understanding the genesis and localization of ore deposits", whereas Edwards and Atkinson attempt to "redress the balance between theory and practice" by focusing on "practical implications of the geological setting and genetic models of particular ore deposit types". Guilbert–Park, with over twice as many pages, would qualify as a reference book if the author-laden index could be improved. The difference in length between the two books reflects the amount of introductory material that precedes the ore-deposit descriptions, with only 17 pages in Edwards–Atkinson versus 306 pages in Guilbert–Park.

Both books wrestle with the perennial problem of classifying the wide diversity of mineral deposits and both adopt field-based schemes. Guilbert and Park organize their book around geological settings, whereas Edwards and Atkinson use the 'model' approach common in modern exploration thinking. The contents of both books reflect these classification schemes as well as the authors' backgrounds, with the Guilbert–Park system providing a more comprehensive, flexible coverage. Important deposit types, such as epithermal vein, sediment-hosted gold and magmatic iron deposits, are found only in Guilbert–Park, whereas sedimentary exhalative deposits are better covered in Edwards–Atkinson. Deposits of recent interest, such as hot spring and detachment gold, are missing from both books. The classification of some deposits, such as volcanogenic massive sulphide under the "Magmatic Hydrothermal" class in Edwards–Atkinson, and Mississippi Valley-type lead–zinc and Athabasca-type uranium under "Epigenetic Deposits of Doubtful Igneous Origin" in Guilbert–Park, is confusing at best.

The main shortcoming of both books is the degree to which they integrate their approach to economic geology into the deposit descriptions. Edwards and Atkinson's persuasive call that more attention should be paid to the role of mining, metallurgical, economic and political factors in economic geology, is not matched by their coverage of these topics in the short introduction. The discussions of exploration-related aspects at the end of each chapter are also too short and generalized to be of use to students. Numerous topics ranging from middlings to induced polarization need to be described and related directly to the deposits if the approach is to succeed. Guilbert and Park do a better job of covering the geochemical and other research aspects of economic geology, with the exception of solubility controls on specific metals and minerals, but they do not use this information adequately in the descriptive sections of the book.



W. K. Bilodeau

Geological patterns — colloform banding in a sample of malachite from Zaire. The picture is reproduced from the book by Guilbert and Park, reviewed here.

In spite of these shortcomings, both books contain a great deal of worthwhile material, are well presented, with clear illustrations and complete references, and would serve as satisfactory texts. Guilbert–Park would probably be the first choice of most geologists because of its much greater coverage, but if economic geologists are ever to stop being hired, and fired, by engineers, accountants and lawyers, they should also read Edwards–Atkinson. ☐

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World appearance

Edward Derbyshire

Earth's Changing Surface: An Introduction to Geomorphology. By M.J. Selby. *Clarendon: 1986. Pp.607. Hbk £28, $39.95; pbk £12.50, $24.95.*

Over 20 years ago, Professor David Linton confessed that "geomorphology is, and perhaps always will be, one of the more difficult of the physical sciences". The root of this difficulty lies in the mixed conceptual base of the subject, which draws upon both historical analysis using a deductive framework, and functional studies which consider the behaviour of Earth materials and the processes acting upon and within the Earth's surface skin. The task of compiling a textbook to cover the whole field is thus one which demands deft handling of concepts and examples, so as to illuminate the explanations rather than give undue emphasis to the problems posed by the subject's diverse approaches, techniques and objectives.

On the face of it, much of *Earth's Changing Surface* is organized along traditional lines. There is an initial chapter on basic concepts, followed by four chapters on tectonics and volcanism as landform-shaping agencies, one on the properties of Earth materials, and nine using the familiar framework provided by the principal Earth-surface processes. The presentation of the first chapter is distinctive yet somewhat staccato: mention of the energy sources for landscape change, inheritance of landscape features, and time-scales precedes the definition of geomorphology which appears in a short account of the objectives and history of the subject a little further on.

The placing of a long section on climatic geomorphology after the historical account and before the brief treatments of equilibrium, geomorphic systems and applied geomorphology ("People as Geomorphic Agents") might bemuse the beginner for whom the book is intended. In fact, the progression is logical, but it could have been more plainly signposted. In contrast, the chapters on the main features of the Earth, global tectonics and structural landforms provide a clear introduction to global processes and their relationship to major landforms and structural features of the Earth, giving this subject an emphasis which has been called for in general geomorphology texts for some years. These chapters provide a wide range of basic information, from the classification of the igneous rocks to large-and small-scale structures, their resultant landforms and drainage patterns. Professor Selby effects the shift in scale down to weathering, hillslopes and the other process realms by inserting a chapter on the behaviour, strength and resistance of rock, soil and water.

Many users of this book will judge it on the nine chapters devoted to process–landform relationships. I found them to be up to date, well illustrated and generally well balanced in content: the standard is appropriate for the beginning student with some basic science background. There is a minimum of mathematics, but, when used, it is fully supported by an explanation in the text.

Reaction to the last five chapters, which deliberately address broader issues, will vary. Their basis is largely climatic, yet 15 chapters separate them from the discussion of the principles of climatic geomorphology in Chapter 1. The chapter on Ice Ages can be read as a free-standing essay; but to do justice to this closely written section, the student reading the succeeding chapter on planation and those on the landforms in the major climatic belts will require the guidance of a good teacher to ensure cross-reference to the earlier discussions of process relationships.

Profesor Selby thus presents his subject matter in an interestingly different way. There is much to prod students into reading further, the last four chapters taking them beyond the introductory stage. This is an altogether impressive textbook, which will also serve well as a reference work for those requiring up-to-date views on landforms and landform change. □

Edward Derbyshire is Professor in the Department of Geography, University of Leicester, Leicester LE1 7RH, UK.

Burning issues

Ian Fells

Renewable Energy Resources. By John Twidell and Anthony D. Weir. *E. & F.N. Spon/Methuen: 1986. Pp. 439. Hbk £30, $65; pbk $29.*

Learning about Energy. By David J. Rose. *Plenum: 1986. Pp. 506. $59.50.*

Renewable Energy Resources is designed to bridge the gap between descriptive reviews and specialized engineering treatises. It is intended for undergraduates in physical science and engineering and largely hits the mark, leaning towards the fundamentals of the subject with satisfying mathematical descriptions of the various phenomena: wind and wave power, solar heating and so on.

I suspect, however, that the innumerate renewable energy enthusiast, who feels instinctively that what is natural is good, will make no headway with this book, despite the sympathy of the authors. They point out in their preface that they enjoy the subject and hope their readers will also, and that practitioners must put on their rubber boots or sun hat and move outside. They might have added that a familiarity with vector analysis is also essential.

All of the principal renewable energy sources are analysed, and chapters on fluid mechanics and heat transfer are included to aid understanding. Practical details are largely absent, although a traditional charcoal kiln is described. There are quite searching sets of questions at the end of each chapter. Altogether, this is a useful textbook; my only criticism is that the word 'economics' does not appear in the index, and some analysis of costs would have put renewable energy resources into perspective alongside more traditional fossil fuels.

No such criticism can be levelled at *Learning about Energy*, which "deals with energy as more than technology or economics or any other specific parts. It deals with energy as the fabric of civilization". The author's claim is substantiated by the arrangement of his material. The first two chapters are a general discussion about energy in a social context, in tune with his conviction that "energy is a means and not an end and stands for a series of activities engaged in by society in accordance with a variety of sometimes-conflicting goals". He chooses acid rain and the greenhouse effect as two examples of large burdens laid upon us by energy use. It follows that energy should be used effectively, and after explaining how this should be done the topics of oil, gas, coal and nuclear and solar energy are considered in some technical depth. Nuclear energy is picked out for particular examination; included here is an account of comparative risk assessment.

A puzzling omission, common to many books of this kind, is of any detailed treatment of the combustion process. Some 90 per cent of the world's energy is derived from burning some kind of hydrocarbon-based fuel, and the science and technology of combustion must be properly understood if it is to be carried out effectively. This book, for example, relegates combustion of pulverized coal in power stations to two paragraphs, although 15 pages are given to the structure and mining of coal. Combustion of oil and gas is not discussed at all. On the other hand, the economics of energy usage are well presented.

The treatment is entirely US-orientated, much of it in what the author quaintly describes as "Old English" units. The approach is mature and the book will be particularly appropriate for graduate courses. □

Ian Fells is Professor of Energy Conversion in the Department of Chemical Engineering, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, UK.

In and out of the laboratory

Maurice Rigby

Experimental Physical Chemistry. By G. Peter Matthews. *Clarendon: 1986. Pp. 495. Hbk £32.50, $49.95; pbk £16, $29.95.*
A Textbook of Physical Chemistry. By A.S. Negi and S.C. Anand. *Wiley Eastern: 1986. Pp. 972. Pbk £10.50.*
Introduction to Physical Chemistry. By M.F.C. Ladd and W.H. Lee. *Cambridge University Press: 1986. Pp. 347. Hbk £27.50, $49.50; pbk £9.95, $19.95.*

THE changing face of physical chemistry is reflected in the 59 experiments selected for inclusion in Peter Matthews's book, the declared aim of which is to provide a practical course related to the material currently taught in degree courses. There is a good range of traditional experiments (including one or two venerable examples, such as the Dumas method of vapour density determination), but the results of recent technical innovation are well demonstrated in exercises such as laser Raman and Fourier transform infrared spectroscopy, microprocessor controlled *p*H titrations and molecular dynamics 'experiments' by computer simulation.

The book is in three parts, "Equilibrium", "Structure" and "Change", and is further divided into eight sections of grouped experiments. Each section has an introduction, putting the experiments in context, and each exercise is preceded by a summary of the appropriate theory and by a description of apparatus, experimental procedure and so on. Technical notes provide information for supervisors and technicians, including a guide to cost and complexity. Finally comes a list of selected references.

Among the experiments there are some interesting and unusual examples, such as the measurement of the surface tension of liquid oxygen and a study of fluorescence quenching by electron transfer to metal ions. Equilibrium electrochemical measurements are perhaps favoured at the expense of dynamic studies (represented only by a polarography experiment) and the comparatively large number of exercises involving vacuum lines will tax the technical resources of some departments; indeed, it is likely that many departments will be unable to offer more than a limited selection of the experiments involving expensive or complex equipment. Nevertheless, there are numerous exercises which make more modest demands, and teachers planning a laboratory course, or looking for new experiments to add to an existing course, will find much of interest here.

In contrast to Matthews's book, there is little indication in the other two volumes reviewed here that much progress has occurred in physical chemistry during the past two or three decades. *A Textbook of Physical Chemistry* by Negi and Anand was written for degree students in Indian universities. The book follows a conventional sequence. It is characterized by the painstaking development of even elementary algebraic expressions, and offers little in the way of physical insights. There are many worked examples and large numbers of exercises at the ends of chapters, but the general standard of presentation is poor, with inconsistent notation and many typographical errors, by no means all of which appear in the six-page errata. The level reached is below that required for degree work in Britain or the United States, and the overall impression is of a rather old-fashioned text. For example, one must question the value of a nine-page exposition of the Bohr–Sommerfeld theory and four pages dealing with the parachor in a book that devotes less than thirty pages to the whole of molecular spectroscopy.

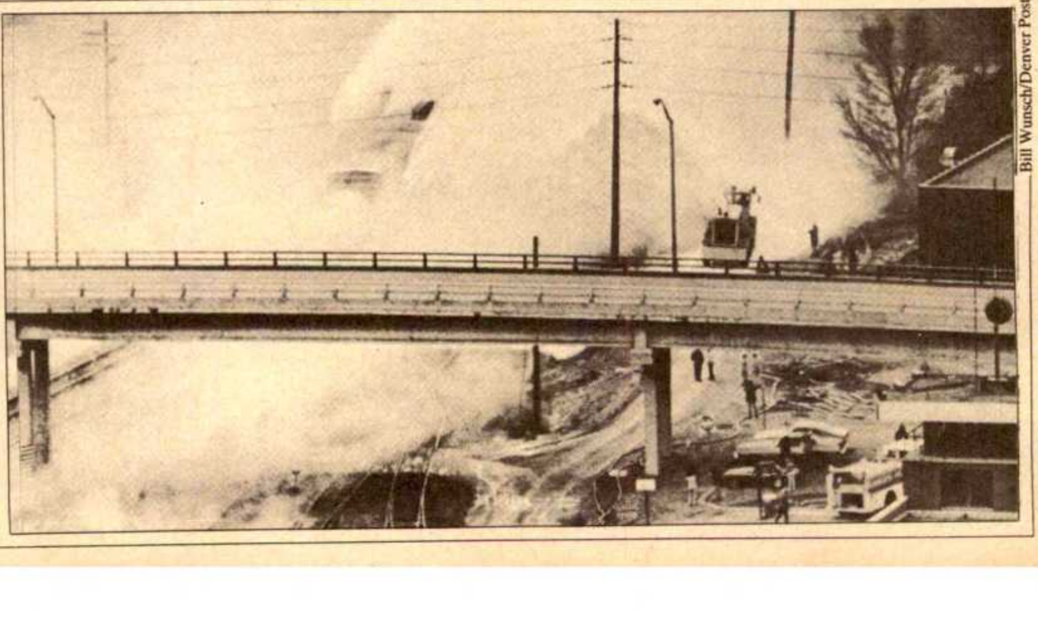
It seems unlikely that this book will offer serious competition to established texts, such as Atkins, Barrow, Castellan, Moore and others. It certainly represents remarkable value in terms of pages per pound, though the quality of paper and typesetting reflect the price.

Ladd and Lee's *Introduction to Physical Chemistry* declares itself to be aimed at students in the first year of a chemistry degree course. It is developed from the same authors' *Modern Physical Chemistry* (Penguin, 1969) and parts of the text are identical with the earlier work. Many beginning students may well find the conventional single-volume comprehensive textbook daunting and would welcome an introductory text of this sort, but it is unlikely that the present book will meet their needs. The choice of material is unbalanced, with almost fifty pages devoted to the solid state but fewer than ten on the whole of spectroscopy, while the clarity of exposition leaves much to be desired. The account of atomic and molecular structure is extremely confused; the uncertainty principle is presented as arising from attempts to apply the Bohr theory to systems containing more than one electron, and the same theory is said to be unable to account for the bonding in methane and ethene. It will surprise most chemists to learn that this was an objective of the Bohr model!

The description of bonding theories is also inadequate, and matters do not improve greatly in the chapter on thermodynamics. Here the exposition is characterized by numerous imprecise (and occasionally incorrect) statements, and the whole section will increase rather than dispel the confusion often experienced by newcomers to the subject. In brief, one must regretfully advise students seeking a good introductory textbook of physical chemistry to look elsewhere. ☐

Maurice Rigby is a Lecturer in the Department of Chemistry, King's College London (KQC), Kensington Campus, Campden Hill Road, London W8 7AH, UK.

Chemical confrontation — a snowblower pumps sodium carbonate onto a serious spill of nitric acid near Denver, April 1983. This is a popular picture; it appears both in the seventh edition of John R. Holum's Elements of General and Biological Chemistry *(Wiley) and the second edition of* General Chemistry, *by Donald A. McQuarrie and Peter A. Rock (W. H. Freeman).*



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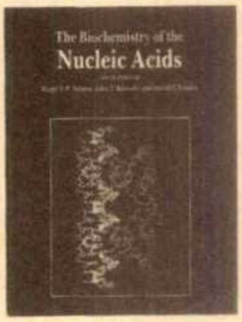

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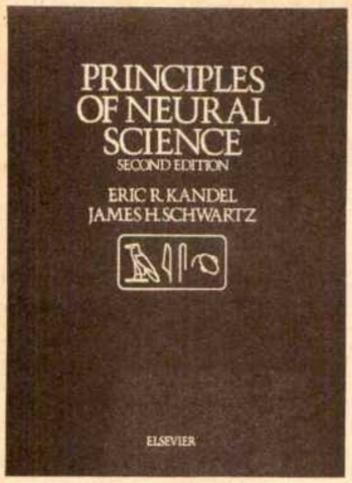

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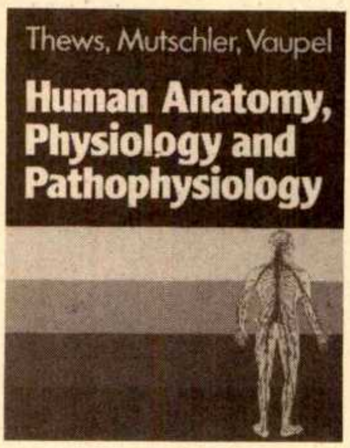

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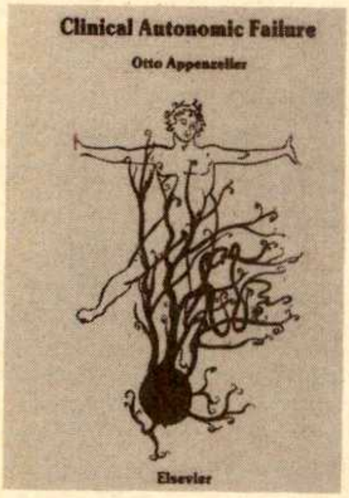

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FROM ELSEVIER

Light for organic chemists

Andrew Gilbert

Introduction to Organic Photochemistry. By John D. Coyle. *Wiley: 1986. Pp.176. Hbk £26.25, $31.95; pbk £8.35, $13.55.*
Organic Photochemistry, 2nd Edn. By J.M. Coxon and B. Halton. *Cambridge University Press: 1987. Pp.243. £37.50, $69.50.*

IN THE late 1960s and early 1970s, the rapid development of organic photochemistry resulted in the appearance of a number of introductory textbooks on the subject. In the past ten years, however, reflecting the lack of commercial exploitation of the area, few organic photochemistry texts at any level or degree of specialization have been published. The new and widespread interest in synthetic applications should lead to a renaissance of the subject, and the time is thus ripe for the writing of new texts which take account of recently accumulated knowledge of photoinduced organic reactions.

The books under review are both intended as introductions to organic photochemistry and they achieve this purpose most satisfactorily. Both are well-written and up-to-date with clear presentation of well-chosen material. But although the authors cover much the same ground, and at an appropriate level for undergraduates, they have organized their subject matter rather differently.

Most organic textbooks present the material according to functional group and this is the approach successfully used by Coyle. Thus, following the first chapter which outlines the general features of photoreactions, there are chapters dealing with the photochemistry of alkenes and related compounds, carbonyl compounds, and arenes, while the final chapter has sections on the photoreactions of all other chromophores. Coxon and Halton's book, the successor to a volume published in 1974, has a similar first chapter to that of Coyle, but the photoreactions are presented quite differently. There are two chapters on intramolecular reactions of the alkene bond and of the carbonyl group, and then a chapter on intermolecular cyclo-addition reactions of alkenes, carbonyl compounds and arenes. The final chapter deals with oxidation, reduction, substitution and elimination processes. Inevitably the last chapter in both books is somewhat of a mixed bag, but personally I prefer presentation by chromophore type if only because the enormous amount of carbonyl photochemistry is in one chapter rather than spread through three. Having noted this, it must be said that within the confines of their chosen approach Coxon and Halton do discuss and illustrate the material in a readily understandable manner.

Coyle's book is intended for a general readership having a knowledge of basic organic chemistry, and the author considers that the theoretical bases of pericyclic reactions and Woodward–Hoffmann aspects are more appropriately dealt with in a general text. In contrast, Coxon and Halton intermingle explanations of these latter topics with the organic photochemistry; while this will appeal to undergraduate and postgraduate students, it does tend to break up the readability of the text for those who simply wish to gain a general appreciation of the photoreactions of organic compounds.

The two books also differ in the way that help is given to the reader to pursue the subject in more detail, although both approaches are satisfactory. Coxon and Halton give specific references to review articles and the primary literature throughout their text, whereas Coyle provides a list of further reading at the end of each chapter and adds a useful note to each item outlining the subject matter covered. Neither book includes questions and problems, which is surprising but not a drawback; in my view such sections are rarely used by students.

Newcomers to organic photochemistry will benefit greatly from reading either of these texts. Both provide a sound introduction to the subject, and prospective buyers will have to make their own choice according to cost and their personal preferences for the detailed content and organization of the material. ☐

Andrew Gilbert is Reader in Organic Chemistry in the Department of Chemistry, University of Reading, Whiteknights, Reading RG6 2AD, UK.

Diverse problems

Selby A.R. Knox

An Introduction to Organometallic Chemistry. By A.W. Parkins and R.C. Poller. *Macmillan, London/Oxford University Press, New York: 1986. Pp. 252. Hbk £22.95; pbk £9.95, $18.95.*

THE organic chemistry of metals is a huge field, extending from lithium at one end of the Periodic Table, through the p- and d-block elements and the lanthanides, to the actinides at the other. One of the great attractions of the subject is the consequent diversity of structure, bonding, synthetic methods, reactivity and applications that it presents. Yet this very diversity makes any attempt to cover the whole field in a relatively short book, as here, a difficult one. In also aiming to provide both an undergraduate textbook, and specialist information for research workers, the authors made their task even harder. The result is disappointing.

Because the fundamentals are so different, the organometallic chemistries of main group and transition elements are normally taught separately, but as closely related areas. The authors have chosen an integrated approach but this often demands an artificial organization of the material under generalized, unhelpful headings in a forced attempt at harmony. Thus, for example, we find quadruply bonded divanadium compounds bracketed with antimony and bismuth alkyls, lithiation of arenes and *ortho*-metallation by transition elements taken together, and the synthesis of alkylidene complexes under "Reaction of Organometallic Reagents with Unsaturated Compounds".

But it is the superficial treatment of the basic aspects that is most disturbing. We learn only that the 18-electron rule is empirical (this is even "stressed") and are given no understanding of its basis or the reasons for the existence of 16-electron compounds, so important in the application of organometallics to catalysis. Within the 250 pages, only three are devoted to the vital processes of oxidative-addition, reductive-elimination and migratory insertion, while β-elimination is hidden in sections entitled "Reaction of Organometallic Reagents with Metal Halides" and "Adducts of Organometallic Compounds"; the account of stereochemical non-rigidity warrants four pages. One even searches in vain for any thorough discussion of the way in which the reactivity of an alkene can be modified through coordination to a transition metal. The classic story of cyclobutadiene stabilization by complexation merits only a brief mention, and the wider issue of such stabilization of unstable organic molecules and the reasons for it are not addressed.

Irritatingly, the term 'insertion' is used throughout the book for reactions which are now known to proceed in reality by migration, and although this is explained on one occasion the many diagrams which involve this pervasive process are wrong mechanistically in depicting an insertion path. This confusion is most apparent in the chapter "Catalytic Applications of Organometallic Compounds", where hydroformylation is also described in an often inaccurate fashion.

In attempting too much, and thereby being driven to brevity and superficiality, the authors have produced neither a particularly good introduction to the field nor one which serves as a valuable source of information. As it stands, the book will not appeal to a wide audience. ☐

Selby A.R. Knox is a Reader in Inorganic Chemistry in the School of Chemistry, University of Bristol, Bristol BS8 1TS, UK.

An atmosphere of accessibility

Robert A. Duce

Air: Composition and Chemistry. By Peter Brimblecombe. *Cambridge University Press: 1986. Pp. 224. Hbk £25, $39.50; pbk £8.95, $14.95.*

Air: Composition and Chemistry is part of the Cambridge Environmental Chemistry Series, the goal of which is to provide a central core of texts for undergraduates and first-year graduate students in environmental chemistry. The early chapters briefly summarize physical properties of the atmosphere and discuss the chemistry of the lower atmosphere, including sources, sinks and residence times for atmospheric species, atmospheric photochemistry, aerosol chemistry and cloud/precipitation chemistry. Later chapters cover sources and effects of air pollution, and the chemistry of the upper atmosphere and of planetary atmospheres. While used sparingly, references are up-to-date, with many published in 1983 or later, and several review articles or books are listed in each chapter for further reading. No problem sets are given.

A book of 200 pages could not hope to cover such a broad range of topics in great depth. Therefore the author generally follows a non-rigorous, descriptive approach, which suggests that the students using the text are not necessarily expected to have a strong scientific background. In most sections this approach works well, and the author has generally succeeded in making the topics relevant and interesting to non-specialists in atmospheric chemistry. However, at times simple explanations are combined with rather complicated mathematical equations. Often these equations are not used nor their derivations presented, for example the complex equations for calculating plume dispersal and Rayleigh and Mie scattering. Such terms as vibrational and rotational changes, spin conservation, terpenes and Gaia hypothesis are used with no explanation, which may also be confusing to non-specialists.

There are several notable omissions. There is little mention of aerosol particle residence times, and the discussion of indoor pollution does not address current concerns about radon and asbestos. After stating that the atmosphere is in steady-state chemically, it is pointed out that the concentrations of several gases are increasing globally, including carbon dioxide, methane and nitrous oxide; however, the potential impact of the increase of these and other trace gases on future climate and on the oxidizing capacity and photochemistry of the global atmosphere is only hinted at.

The book is refreshingly free of typographical errors and will be a very useful supplemental text for atmospheric chemistry sections of undergraduate environmental chemistry courses. It gives a broad and generally well-integrated picture of our current understanding of atmospheric geochemistry and air pollution chemistry. But I would not recommend it for an in-depth undergraduate or graduate course in atmospheric chemistry. ☐

Robert A. Duce is Professor of Oceanography and Director of the Center for Atmospheric Chemistry Studies, University of Rhode Island, Narragansett, Rhode Island 02882, USA.

THE MANAGEMENT OF AIDS PATIENTS

Edited by DAVID MILLER, JONATHAN WEBER and JOHN GREEN

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Chain effects

Paul Calvert

Introduction to Physical Polymer Science. By L.H. Sperling. *Wiley: 1986. Pp.439. $39.50, £38.05.*

A TECHNOLOGICAL proverb says that whenever you open a can of worms the only way to get them all back in is to use a larger can. It is the same with textbooks, where the need to update some topics raises the difficult question of what to expand and what to drop.

The author of this book, L.H. Sperling, is at Lehigh University where physical polymer science is a final-year undergraduate course offered along with polymer chemistry and practical courses. If we take our original can of worms as being Billmeyer's *Textbook of Polymer Science* (Wiley; 3rd Edn, 1984), then Sperling has dropped the polymer chemistry, as his title implies, and also the survey of commercial plastics and processing methods. Mechanical properties are treated in the final chapter but in a rather sketchy way. In return for the omissions we get material on neutron scattering, chain packing and folding, and reptation, as well as detailed accounts of crystallization kinetics and glass-transition theories.

The approach is often original, but the development of the subject matter is clear and progressive. After an introductory survey, chain configuration is discussed with reference to NMR and IR spectroscopies. The chapter on molecular weights covers osmometry, scattering methods, viscosity and gel permeation chromatography, and also neutron scattering. Solution behaviour is covered from the standpoint of phase separation and polymer blends.

The amorphous and crystalline states are treated on the basis of neutron scattering data, and of packing models, and there is a thorough description of crystallization kinetics with nucleation theory and chain folding. Reptation, too, makes an appearance here.

The glass transition is dealt with in some detail but the treatment is fairly traditional; likewise, the chapter on rubber elasticity. The discussion of viscoelasticity and flow is rather disappointing, in that it concentrates on springs and dashpots where I would have liked to see more of the Rouse model and reptation.

In my view the distinctive characteristics of polymers are chain configuration, the random coil and entanglements. Sperling does a good job on the first, treats the second adequately and omits the third. Glass transitions and crystallization are not peculiarly polymeric but must be dealt with in polymer physics because most students do not see them elsewhere. In such a course it is easy to try to cover so much that nothing is done in depth, and for this reason I favour the space given to theories of crystallization and the glass transition. ☐

Paul Calvert is a Lecturer in the School of Chemistry, University of Sussex, Brighton BN1 9QJ, UK.

- Recently published by Wiley Eastern is *Polymer Science*, by V. R. Gowariker *et al.*, a 500-page textbook which covers all aspects of the subject. Price is £38.50, $39.95.

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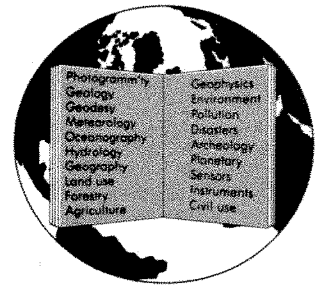

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What's the use of understanding?

K.A. McLauchlan

NMR in Chemistry: A Multinuclear Introduction. By William Kemp. *Macmillan, London: 1986. Pp.240. Hbk £25; pbk £11.95.*

THIS is another attempt to introduce NMR and its chemical applications to undergraduates, but on a wide front. Basic chapters of the book are concerned with fundamentals, spectrometer design, 'advanced' theory, multi-pulse and computational methods and dynamic aspects, whilst individual chapters are devoted to spectra from ^{1}H, ^{13}C, ^{19}F, ^{31}P, ^{14}N and ^{15}N, ^{17}O and 'other' nuclei. A particularly bizarre chapter covers background physics, and provides a 'biography of NMR' which includes those unlikely magnetic resonance scientists Arrhenius and Van't Hoff but misses out some of the major figures. Tables of reference data are provided on ^{1}H and ^{13}C spectra (and the Greek alphabet!).

It is a self-satisfied book, the closest approach I have yet seen to a coffee-table textbook. Content has yielded to presentation and I should resent paying good money for several half-empty pages, pictures of archaic equipment and gyroscopes, and photographs of the periodic table (if it existed) in 1808. In a postscript of great banality entitled "Nocturnal Processes" (on which title I restrain myself from commenting), the author is quite open about his philosophy. Paraphrasing its content, the idea is that one does not have to understand a subject to use it — not an attitude I immediately sympathize with, although it might occasionally be justified.

However, even if students are to use NMR without understanding it, what they are told about it must be correct and the material must be presented by someone who does understand it, in depth. The author displays too shallow a knowledge to guide others, as is shown by many infelicities. For example, spin-lattice relaxation is said to be caused by electric dipole interactions; remarks made about quadrupolar broadening, contact and pseudocontact interactions and so on are largely incomprehensible; and many of the basic ideas of the origins of the NMR parameters are stated in a needlessly confusing manner. This is rather a pity, for at times the author's approach works quite well. As it is, the book cannot be counted a success. □

K.A. McLauchlan is a Lecturer in the Physical Chemistry Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QZ, UK, and a Fellow of Hertford College, Oxford.

Roundly efficient — one of four incubators employing the 'rolling bottle' system for the production of monolayer cells at the Istituto Zooprofilattico Sperimentale, Brescia, Italy. The picture is reproduced from Basic Biotechnology, *reviewed below.*

Bugs and brass

Stephen Oliver

Basic Biotechnology. Edited by John Bu'Lock and Bjorn Kristiansen. *Academic: 1987. Pp.561. Hbk £57, $85.50; pbk £20, $29.95.*

BIOTECHNOLOGY is a diverse subject with vague boundaries. It is not just recombinant DNA technology, but includes chemical and control engineering, microbiology, biochemistry, chemistry, instrumentation science and genetics. The assembly of a text which covers the basics of so vast an area presents a formidable problem. Many previous attempts have resulted in huge, multi-volume compendia which no student and few libraries can afford. This book attempts to take a hard-nosed (or, as the editors would have it, "brass-nosed") approach and strips the subject down to its essentials.

This is achieved by dividing the book into two parts — "Fundamentals and Principles" and "Practical Applications". In Part I, authors from the individual disciplines each give accounts of the basics which relate to biotechnology. These provide an excellent grounding for students with a background in the biological sciences; kinetics, thermodynamics and mathematical modelling are all well explained. They are not so appropriate for engineering students, however, because the principal actors in the drama appear to have left the stage. We learn little of the microorganisms themselves; there is nothing on cell structure, growth habits or life cycles. The engineering student is presented with a masterly account of microbial metabolism and will learn, for example, of all the mitochondrial pathways without actually being told what a mitochondrion is. Similarly, topics such as gene regulation and the cell cycle are given scant coverage.

In Part II, the intention is to demonstrate how the various disciplines are integrated to produce efficient industrial processes. In some chapters, for instance that on biotransformations, this works well. In others, the authors have tried to be too comprehensive, and consequently have produced pieces which tend to be catalogues. The last three chapters, on genetic engineering, and animal and plant cell culture, are rather more forward looking than the rest. But even here there is an emphasis on what has been firmly established and successfully implemented. In the contribution on genetic engineering, for instance, there is no discussion of site-directed mutagenesis.

Each chapter provides a reading list rather than a full bibliography. The references given are to reviews and books published in the 1970s or very early 1980s, which must themselves have been dated when they appeared, and pointers to the current literature would have been welcome.

This text, then, is indeed basic and brass-nosed. It is also authoritative, and will provide students from the biological sciences with a firm foundation in biotechnology. I will direct them towards it but, at the same time, will point out that if they are to contribute to the future of the area they would do well to look up into the blue skies, and check that there is enough brass around to back them. □

Stephen Oliver is Director of the Manchester Biotechnology Centre, UMIST, PO Box 88, Manchester M60 1QD, UK.

Developing concepts of biology

David R. Garrod

Developmental Biology. By William F. Loomis. *Macmillan, New York/Collier Macmillan, London: 1986. Pp.405. $33, £22.*

"O THIS learning! What a thing it is!" And how may it best be accomplished? In this textbook for students of developmental biology, Loomis adopts an interesting approach that may be described as a tall and thin. In 400 pages, he takes the reader from the most elementary rudiments to much more advanced and up-to-date aspects of the subject, going into depth only with a few specific samples.

After a brief historical introduction, the text is divided into three sections: "Mechanisms", "Processes" and "Chosen Systems". The first deals with genetic and physiological aspects of cellular differentiation, the second is really descriptive embryology, and the third considers in more detail particular topics (such as limbs and pattern) or well-studied organisms (such as *Dictyostelium, Drosophila* and *Caenorhabditis*). Together with the "specific examples" given at the ends of the earlier chapters, the later chapters give the book its strength: in general they are good and readable. The other aspects, particularly the descriptive embryology, have been frequently covered elsewhere, and often rather better. Among the specific examples, there is a distinct bias towards the control of gene activity and differentiation, which are well done, while other developmental topics, such as the control of morphogenetic movements, cell motility and adhesiveness, have much less emphasis.

The text is clearly and straightforwardly written, and is easy to follow. The illustrations, however, are for the most part the same old ones that could now justly be described as hackneyed; they may be appropriate for a textbook, but they do not fill one with enthusiasm. Learning aids are provided in the form of tables giving guides to the subjects covered (really a supplement to the index), helpful study questions and reading guides, and brief summaries in each chapter which are rather less useful.

Developmental Biology is certainly worth considering for teaching purposes, especially if one is looking for a book that will take the student through the various levels of study. Loomis has made a brave attempt at a difficult job, but perhaps one ought to conclude that, as with virtue, "There is no road and ready way to" learning and teaching developmental biology. ☐

David R. Garrod is Senior Lecturer in the CRC Medical Oncology Unit, University of Southampton, Southampton General Hospital, Southampton SO9 4XY, UK.

Look to tomorrow

J. Robert S. Whittle

Gene Activity in Early Development, 3rd Edn. By Eric H. Davidson. *Academic: 1986. Pp.670. $49.50, £41.50.*
Genetic Analysis of Animal Development. By Adam S. Wilkins. *Wiley: 1986. Pp.546. £66.95, $69.95.*
Foundations of Developmental Genetics. By D.J. Pritchard. *Taylor & Francis: 1986. Pp.372. Hbk £33, $63; pbk £14.50, $33.*

A GREAT deal hangs on the outcome of the genetic analysis of developmental systems. Evolutionists expect to learn about the type and magnitude of functional changes in genome structure that accompanied diversification of animal forms; neurobiologists hope for coherent ideas about the generation of nervous systems to parallel the sophistication with which they can examine functional connectivity in neuronal circuitry; protein and membrane biochemists want to be sure that they are facing up to the most relevant facets of behaviour of molecular assemblies in cells; and theoretical biologists will be interested to know whether there will be enough information to construct an algorithm or a generative model, and whether it is still worth importing complex system analysis. As well as offering a powerful expansionist technology, molecular biology has brought to genetics the attention of these neighbouring disciplines because the promise of a general understanding of developmental processes is in the air.

The promise of resolution may prove to be false, but how will these three books encourage a student to understand the excitement and anticipate progress in this area? They are not really competitors. The declared and achieved scope of each of them is different, their styles are distinct and they may epitomize different philosophies.

Davidson is a fundamentally recast and expanded third edition of his 1968 book (then of only 375 pages). I agree with his own description that it contains "more molecular and genetic evidence that is of *biological* import than did its predecessor". It is a meticulously prepared heavyweight review, clearly written but with the density of citation and numerical detail that are the stuff of reference works. Having seen how well it treats the area familiar to me, I regard it as an invaluable insurance against having to read more than a fraction of the 1,800 or so references cited. It would make a tough but rewarding Bible for a one- or two-semester series of graduate discussion seminars. It is no textbook but comparison with the other two volumes illuminates all three.

Taking only the "genetically tractable systems" *Drosophila, Caenorhabditis* and *Mus*, in his book Wilkins gives a masterly presentation of the internal logic and working methods that geneticists use to 'dissect' developmental processes. His style encourages participation in the deliberation within each case study, so that the reader appreciates the experimental strategies and judgements involved as well as the specific conculsions. Wilkins is definitely in charge of the discussion, dealing sensibly, as Davidson does discreetly, with the recurrent metaphor of the genetic programme and choosing an operational definition of determination to avoid having to satisfy legion other armchair definitions. If we don't stop and think occasionally, we become kleptomaniac about data, but as a geneticist Wilkins also knows there is a constant threat that discussion may descend into metaphysics. He avoids it. The detail in his expositions might well be from the research seminar or the conference coffee break, and provides a logical and explicit continuity that is frequently absent from research papers.

Pritchard has broader horizons. First, he draws material from more experimental systems, in particular from vertebrates (his own research is on transdifferentiation in the vertebrate eye). He raises global issues of adaptation and evolution in developing systems, discusses genetic assimilation and atavism, and repeatedly encourages the reader to wider perspectives. He then offers an interesting synthesis in Chapter 14, under the title "Principles of the Adaptive-Inductive-Genetic Model", that attempts to encompass many of these classic case studies. The order of topics is unusual, and deliberately so, moving from 'classical' embryological phenomena to differentiation and then on to several chapters on the machinery of gene expression (RNA, chromosomal proteins, transcription) and then to growth and morphogenesis.

In a course on development in 1963, part of my own genetics degree, I recall a bizarre *mélange* of phenomena (serotypes in *Paramecium*, homoeotic mutations, X-chromosome inactivation, genetic assimilation and position-effect variegation) which were supposed to come in handy one day or, as I judge now, become albatrosses for any grand synthesis. I sense that Pritchard might have preferred to enlarge upon some of his material (for example, the Leader cell hypothesis for transdifferentiation) but felt that convention re-

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quired him to take space for groundwork. This, unfortunately, has constrained elaboration of his epigenetic standpoint (he was a student of Waddington and the book reflects Waddington's philosophy). On the other hand, Pritchard provides an introduction to developmental phenomena way beyond the territory of geneticists. It was courageous to introduce recapitulation, perhaps as a hostage to fortune, because we don't know whether this long-standing idea of ontogeny recapitulating phylogeny reflects a common developmental 'syntax' operating in diverse embryos and whether it is worth pursuing.

To be useful today, these texts must be forward-looking. But it is difficult to guess the shape of any grand synthesis until we have assimilated the impact of the currently fashionable tools in investigating gene expression in development. *In situ* probes for transcripts and protein gene products allow us access to the molecular anatomy of embryos; 'foreign' promotion of genes and of anti-sense constructs asks where the *absence* of gene expression is significant; iterative hunts for DNA sequences related to existing cloned genes are short-circuiting mutational dissection, while computer searches of virtual amino acid sequences from clones and sequenced DNA are identifying protein functions (EGF, homoeodomains, serine proteases) in genes previously known only by their mutations; and fission yeast seems the place to examine certain fundamental cell-division functions of the human genome.

The choice is not resolved. Pritchard offers a provocative perspective on developmental systems in the broadest terms but will require additional teaching commentary. Wilkins has the fluency and depth in the current technology and logic used in the three organisms that are going to remain field-leaders for geneticists. And for reference the sections in Davidson on cytoplasmic localization and lampbrush chromosomes are unmatched. Finally, may I record my hope for a more imaginative pricing of a paperback edition of Wilkins. □

J. Robert S. Whittle is a Lecturer in the School of Biological Sciences, University of Sussex, Brighton BN1 9QG, UK.

Spring books

The next book review supplement to appear in *Nature* is Spring books, which comes out in the issue of 30 April.

Among the books to be reviewed are *Time's Arrow, Time's Cycle: Myth and Metaphor in the Discovery of Geological Time* by Stephen J. Gould; *Philosophy and the Brain* by J. Z. Young; *The Communist Party and Soviet Science* by Stephen Fortescue; *Extinction* by Steven M. Stanley; *Never Satisfied: A Cultural History of Diets, Fantasies and Fat* by Hillel Schwartz; and the autobiography and biography, respectively, of Luis Alvarez and Isidor Rabi.

Genetic renewal

D.J. Cove & J.A. Stewart

An Introduction to Genetic Analysis, 3rd Edn. By David T. Suzuki, Anthony J.F. Griffiths, Jeffrey Miller and Richard C. Lewontin. *W.H. Freeman: 1986. Pp.612. Hbk $35.95, £35.95; pbk £22.50.*

Concepts of Genetics, 2nd Edn. By William S. Klug and Michael R. Cummings. *Scott, Foresman & Co, Glenview, Illinois/ Eurospan, London: 1986. Pp.699. $28.76, £17.50.*

THE new editions of these two texts are not radically different from their predecessors. Both adopt a strictly classical approach to the teaching of genetics, dealing with eukaryote transmission genetics and cell division before molecular genetics. In both, mendelism is introduced with Mendel and population genetics is relegated to its familiar terminal position. Klug and Cummings place quantitative genetics where it logically belongs, following the analysis of the inheritance of discrete characters, but their treatment of this subject and particularly of heritability is patchy and not as good as that of Suzuki *et al.*

In view of the central relationship of evolution to genetics, both sets of authors adopt a surprisingly low key approach to the subject. Suzuki *et al.* are particularly coy; only two page references appear in their index, and one of these is to the summary of a new chapter on transposable genetic elements. The summary begins "Nature has devised many different ways of changing the genetic architecture of organisms" and goes on to mention "selfish DNA", but this topic is not alluded to elsewhere in the chapter or in the book. Authors in the United States may feel it necessary to adopt this approach to placate the Creationist lobby, but if this is so it is to be regretted and diminishes the usefulness of their books.

For those who want a traditional approach to genetics, Suzuki *et al.*, with its excellent diagrams and illustrations, remains a strong contender for the textbook of choice. The index, however, is poor. Neither of these books is as useful as a reference work as M.W. Strickberger's *Genetics*, published by Macmillan in the United States and Collier Macmillan in Britain, which went into a third edition in 1985. Strickberger may be less suitable for self-instruction, but on almost every test it emerges as better for reference. Topics as diverse as punctuated equilibria and ribosome binding sites appear only in Strickberger, and most subjects are easily accessible by way of the index. It is principally for this reason that Strickberger will remain the general genetics textbook that we recommend to our undergraduates. □

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Access to theory

Joe Felsenstein

Population Genetics. By Freddy B. Christiansen and Marcus W. Feldman. *Blackwell Scientific: 1986. Pp.196. Pbk £14.80, $19.95.*

Basic Concepts in Population, Quantitative, and Evolutionary Genetics. By James F. Crow. *W.H. Freeman: 1986. Pp.273. Hbk $28.95, £28.95; pbk $15.95, £15.95.*

POPULATION genetics has arguably the most complex body of theory in biology. For that reason the subject has never been easy to explain to biologists, who are often uncomfortable with mathematics and are usually reluctant to concede any role for theory in their science. The rise of molecular biology, with its paradigm of on–off switches, has directed attention away from quantitization, as has the recent scepticism directed at neo-darwinian theory. Yet the molecular data themselves preserve information connecting the genes of different populations and different species. As the tide of molecular sequences rises, and as computers lead increasingly to the quantitization of traditional morphological studies, the need for biologists to apply microevolutionary theory becomes greater.

Fortunately, successive textbooks have made the theory steadily more accessible. The two books reviewed here continue this trend. Both are available as paperbacks and are aimed largely at an undergraduate market. Christiansen and Feldman start with an overture, taking one-third of their book, on the description of variation in natural populations. The many distributions shown give an impression of concreteness which I found refreshing. But inevitably less room is left for the exposition of the detailed structure of the theory of changes in genetic composition of populations. Among the parts of the theory that are done less than justice is the decomposition of genetic variance of quantitative characters.

With smaller type and more pages, Crow's book is nearly twice as long, and proportionally more of it is spent presenting the theory (for example, the treatment of selection occupies 39 pages against 18 in Christiansen and Feldman). Although the writing is lucid, sometimes the explanations would have been clearer with more supporting numerical examples and diagrams; the treatment of the genetics of quantitative characters suffers particularly from their absence. There are many points on which the order and method of presentation are nearly the same in the two books, such as the coverage of linkage disequilibrium. On other matters they diverge, as when Crow cheerfully argues for the adequacy of additive models of quantitative characters, while Christiansen and Feldman warn darkly of their drawbacks.

Crow's book may be directly compared with Crow and Kimura's classic *An Introduction to Population Genetics Theory*, published by Harper & Row in 1970 and re-issued by Burgess, Minneapolis, in 1978. It closely parallels the earlier, more detailed exposition in the order of topics and treatment, but the new book, although shorter, is often actually clearer. Sometimes its status as a condensation of Crow and Kimura becomes obvious, however. For example the Poisson distribution is carefully described in an appendix, but never mentioned in the text.

Both Crow and Christiansen and Feldman make the theory clear and accessible, and in view of the need for population geneticists to do more proselytizing I am very glad that these two books are available. But both of them try to present a large body of theory in too brief a space. It may be hard for the reader to understand how the theory relates to real biological problems when the discussion of applications must be short-changed. If the books are used in a course which provides that context, they can do valuable service. If not, then they seem unlikely, of themselves, to bridge the gap between those who ought to apply microevolutionary theory and those who have been developing it to such heights of complexity. ☐

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USDA

Breeding success — Norman Borlaug, 'father of the green revolution'. The picture is taken from Plant Science *by John Barden, Gordon Halfacre and Dave Parrish, recently published by McGraw-Hill.*

Reproductive ways

Daniel J. Schoen

Plant Breeding Systems. By A.J. Richards. *Allen & Unwin: 1986. Pp.529. Hbk £45, $75; pbk £19.95, $34.95.*

Quantitative Genetics and Selection in Plant Breeding. By Günter Wricke and W. Eberhard Weber. *Walter De Gruyter: 1986. Pp.406. DM198, $79.50.*

THE manifold ways by which plants reproduce has captured the attention and imagination of evolutionary biologists from many backgrounds. Population geneticists, life-history theoreticians and plant systematists are just a few of the students of the subject. Charles Darwin, not usually remembered for his botanical interests, devoted three volumes to the topic. More recently, a number of theoretically minded biologists working on several continents have collectively enriched the study of why there are so many kinds of plant reproductive strategies by posing a simple question which, in essence, asks: when will a mutation which modifies the sexual expression of a plant (or some other component of the reproductive system) be selectively favoured? The predictions of this relatively new body of theory are often stated in straightforward terms, and are helping to transform the study of plant-breeding-system evolution from a largely descriptive endeavour to an empirically rich and exciting branch of biology.

While Richards acknowledges this progress in his preface, his text is built largely around description of the basic features of plant reproduction systems. The topics include floral morphology, plant–pollinator interactions, gene dispersal, incompatibility systems, gender variation, self-fertilization and asexual modes of reproduction. Richards is at his best when discussing the classically botanical aspects of the subject. The chapters on floral diversity, self-incompatibility systems in higher plants and asexual modes of reproduction are thorough and useful reviews. Less thorough and precise coverage is given to the genetic aspects of plant breeding systems, such as the estimation of outcrossing rates and the population genetic consequences of different reproductive systems.

My main disappointment with this book concerns the relatively small role which evolutionary theory has played in organizing the subject matter. Further progress in understanding the diversity of plant breeding systems is likely to come as experimentally orientated evolutionary biologists pay close attention to how models of mating system evolution can best be tested, and when theoreticians appreciate the findings and the difficulties which arise when these models are tested. Richards does address the fitness consequences of different modes of reproduction, but he is not explicit as to how these consquences will influence the selection of mating system modifiers. Nor is he clear on how to estimate male and female components of reproductive fitness, which is so essential in examining the processes that are thought to underlie the selection of mating system modifiers. Many of the critical details, questions and experimental approaches required to test plant-breeding system theory are, therefore, largely left up to the reader to devise.

Turning from the ways in which plants mate under natural conditions, to the ways in which plant breeders selectively mate them for crop improvement, Wricke and Weber's book is a solid addition to the recent harvest of new and revised texts dealing with quantitative genetics. Special consideration is given to practical problems of breeding crop plants with different reproductive and recombination systems. The basic structure of the text (one which I found to be very effective) is separation of the treatments of the quantitative genetic theory of vegetatively propagated, cross-fertilized, self-fertilized and tetraploid crops. Thus, for example, readers with interests in the breeding of cross-fertilized species will find separate sections on the theory of covariances between relatives, the estimation of general combining and synthesizing ability, and mass and family selection, while those interested in self-fertilized species can read separately about the theory and practice of selection with homozygous lines.

The style of presentation is efficient and clean, and the theory is treated in a more quantitative manner than in classic texts such as R.W. Allard's *Principles of Plant Breeding* (Wiley, 1960). The applied nature of the material presented, while not likely to appeal to those teaching quantitative genetics from an evolutionary perspective, makes this a welcome addition to the existing breeding-orientated texts on quantitative genetics. ☐

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Neurochemistry

Fundamentals and Concepts
by
F. Hucho

1986. XV, 326 pages with 250 figures and 25 tables. Hardcover. DM 63.00/US $ 43.45.
ISBN 3-527-25989-9

This textbook describes the molecular basis of the functions of the nervous system. It provides information about important topics of neurochemical research, such as vision, membrane structure and function, electrical properties of nerve cells, synapses and receptors, development, plasticity and learning. The author emphasizes common aspects, outlines the experimental methods and describes model systems that are less complex than the brain, and yet yield relevant information.

From Genes to Clones

Introduction to Gene Technology
by
E.-L. Winnacker

1987. Approximately 590 pages, 330 figures and 35 tables.
DM 120.00/US $ 76.00 (Hardcover).
ISBN 3-527-26199-0
DM 60.00/US $ 38.50 (Softcover).
ISBN 3-527-26644-5

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This book, written by a single author, leads to the frontiers of current knowledge and will help advanced readers in planning and designing their own experiments. At the same time it may be read as an introductory text by undergraduate and graduate students trying to become familiar with the unit operations of gene manipulation.

Fundamentals of Biotechnology

edited by P. Präve, U. Faust,
W. Sittig and D. A. Sukatsch

1987. 751 pages, 197 figures and 107 tables.
Hardcover. DM 295.00/US $ 170.00.
ISBN 3-527-26144-3

This book familiarizes its readers with scientific and technical details that are often left out or taken for granted in multi-volume series on biotechnology. Among its topics are microorganisms, metabolic pathways, products, bioreactors, fermentation processes, biotransformations, production of microbial biomass, environmental biotechnology, and economic aspects. It presents concise working instructions on all technical matters that are possibly encountered in biotechnological routine work. As such it is neither a textbook nor a mere reference work, but meant to be used as a true „handbook" in the working area.

Basic Biotechnology

A Student's Guide
edited by P. Präve, U. Faust, W. Sittig and
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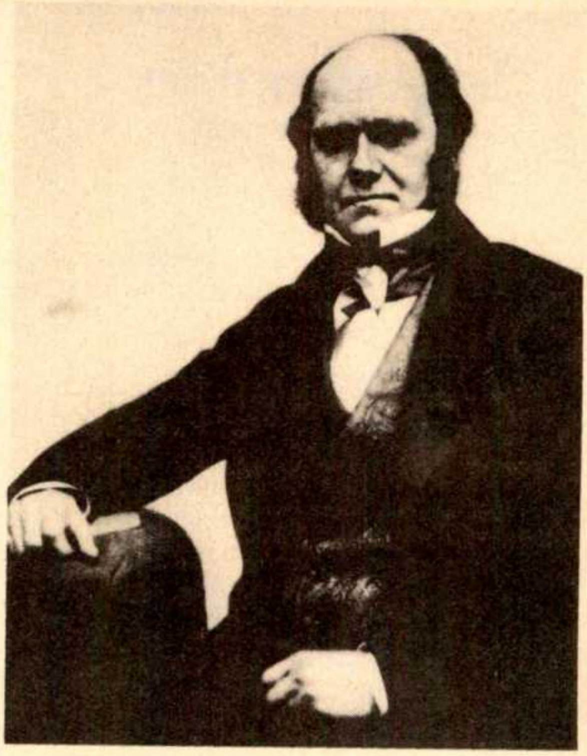

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Revolutionary zeal

Daniel H. Janzen

Plant Ecology. Edited by Michael J. Crawley. *Blackwell Scientific: 1986. Pp.496. Hbk £35, $55; pbk £16.50, $29.95.*

MICHAEL Crawley set out to put together a plant ecology text for the undergraduate, a text that would haul itself out of the musty bog of old world descriptive and aggregate plant ecology and into the chaos of new world evolutionary and ecological biology of plants as individuals and populations. He was generally successful. However, about half of the (very competent) contributors did not fully share or appreciate Crawley's legitimate zeal for this revolution in the way plant ecology is taught in English universities. Many seem to have taken the task on as an "assignment" or as a chance to get a fat review manuscript into print. Were I an undergraduate discovering plant ecology through this book, I think I would be mostly bored. Give the Crawleys of the world the time and resources to write such texts straight through, with a pedagogical, as well as a factual, goal in mind.

The book is built on summary statements, with occasional examples. However, the authors are sometimes not familiar enough with the biology of the examples to avoid over-generalizing. After being told that in animal ecology "the individuals are assumed to blunder about at random", we are informed that "plants compete strongly with only the six or so individuals in their immediate vicinity" (p.253) — someone forgot about competition for pollinators and dispersal agents. And we are told that "strictly speaking, no animals are adapted for pollination" (p.187), when hundreds of species of fig wasp (Agaonidae) are unambiguously adapted for pollination of figs, by any definition of either "adapted" or "pollination".

Again, it is stated that "theory predicts that maximal species richness should occur in moderately resource-poor habitats, habitats that have just enough resources for species survival in the absence of interspecific competition" (p.59). Such a theory is incompatible with the decreasing gradients in plant species richness up the sides of tropical mountains. An entire chapter is based on the concept that "factors causing major reproductive deficits or imposing significant mortality are bound to have evolutionary consequences when there is genotype variation in their effects" (p.336). It would seem that both disruptive selection and phylogenetic inertia were forgotten.

The evolutionary philosophy running through the book is that where there is a selective pressure it is responded to; plant traits are maintained through selection; and those selective pressures are present in the environment of the plant today. All three suppositions are highly suspect in many aspects of field biology. As the preface notes, "Throughout the book we are at pains to stress that our ultimate objective is to measure the impact of the processes we describe on the *fitness* of individual phenotypes" (p.xi). Given that objective, the book would have benefited from an introductory chapter examining why or whether the concept of fitness should be used as the primary point of reference for the plant's interactions with the world. A single phenotype may have extraordinarily different fitnesses in the variety of habitats it occupies and continues to occupy generation after generation. Even when variation appears in the genetic background for that single phenotype, the result *need* not be any realized selection or evolution (though of course it may be — p.251).

Plant Ecology is not horrendously long, and will certainly serve as a good foil for an alert teacher working with well-motivated students. But this kind of teaching, so common in English universities, is rarer in the United States. That leads me to worry about how the book might be used indiscriminately in the New World. □

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Flying and fishing

J. Bryan Nelson

Seabird Ecology. By R.W. Furness and P. Monaghan. *Blackie/Chapman & Hall: 1986. Pp.164. Hbk £19.95, $45; pbk £9.95, $23.*

FURNESS and Monaghan begin their book with an exhilarating, free-style swim through the seas of seabird ecology. Mating systems, colonial breeding, deferred breeding, clutch and brood-size and much else are polished off in a mere 19 pages. It is a tribute to the authors' lucid style and grasp of their material that they nevertheless convey the essentials of the subject (it was presumably a slip to exclude the Sulidae from the families of the Pelecaniformes in the first table). I would have welcomed fuller discussion of social behaviour in relation to breeding ecology, more on the assessment of mate quality (what *is* the evidence that courtship displays are to assess mate quality?) and something on synchrony, the phenomenon of intra-specific interference in breeding colonies and on non-breeders. But one can't have everything in a short account.

At the core of the book are the twin themes of seabird feeding and the interactions of man and seabirds. Almost one third of the contents is devoted to seabirds and fisheries. This selectivity provides a richer texture to the book than a uniform précis, though one does notice the change in writing style from that appropriate to a scientific paper to that used in textbooks. As an adjunct to the emphasis on food and fisheries the authors devote much space to bioenergetics. This, too, is defensible, for this relatively new field generates important computations of the amount of energy which seabird populations take out of the marine environment. Throughout, however, the authors are duly mindful of the pitfalls of using these equation-generated quantities to predict fishery matters. Marine systems are highly complex, involving many interacting species at different trophic levels, and the changes which occur in them will seldom be even qualitatively predictable.

The short review of theories of population regulation is admirable and comes down, in the main, on the side of density-dependent regulation (Wynne-Edwards's new book on group-selection appeared too recently to be mentioned). I myself believe that the regulation of many seabird populations is not density dependent, and the authors, too, include appropriate reservations. Both with regard to population regulation and to the interactions of fishermen and seabirds, the Peruvian anchovy fishery receives much attention. Here I failed to understand how the numbers of the birds concerned could have been unregulated a century ago (p. 45). Niño years have been occurring for much longer than this and were in themselves regulatory. Only once do the authors appear to claim (p.79) that the increase in Peruvian seabirds following a huge, natural reduction was due to the decreased competition for food. Surely, until the relatively recent overfishing by man, the seabirds climbed rapidly back to high numbers because food, irrespective of seabird demands on it, was superabundant?

The section on monitoring the marine environment is timely and comprehensive, dealing with plastics (ingested by seabirds), oil, PCBs, organochlorines and heavy metals. The technique which allows the estimation of mercury levels in feathers could be especially useful because, when extended to museum specimens, it makes historical comparisons possible.

I enjoyed and learnt much from this excellent book. Students, and indeed their teachers, as well as fishery scientists and serious seabird buffs should go out and buy it. □

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Lost in translation

John Eaton

Handbook of Limnology. By J. Schwoerbel. *Ellis Horwood: 1987. Pp.228. £29.50, $53.40.*

SCHWOERBEL'S *Handbuch* has been established as a standard text in many European universities since the first edition appeared in 1971. Limnology is treated in its broad European sense, to include flowing waters as well as lakes. This translation of the fifth edition of 1984 is therefore a potentially useful addition to the range of textbooks available to English-speaking students of freshwater biology.

The book has distinctive emphases on the historical development of the subject and on the influence of catchment on the ecology of inland waters. The examples used draw heavily on the German literature, but although the coverage could be criticized as parochial in this respect, it is no more so than in the many American texts which almost wholly ignore the immense European contribution to limnology. More serious limitations are the lack of tropical studies and the small extent to which literature sources have been brought up to date. For example, in palaeolimnology the most recent citation is 1974, so none of the advances in techniques and knowledge of the subsequent decade are mentioned.

However the worst failing is the poorness of the translation. German sentence construction is often retained, producing a tortuous English style. There are inaccuracies (for example, on p.109 the dinoflagellate *Peridinium* is said to have cilia) and obscurities abound (as on p.27 where resistances to movement through water are translated as "pondering effects"). Together with the frequent misprints, the result is such baffling sentences as the one on p.18, on sources of rivers: "In their termal budget as well as their colonisation there are large differences between these imnological (not hydrological) source categories".

It is a pity that such a potentially valuable book should be spoilt by such easily remediable faults. The *Handbuch* contains fascinating insights and syntheses of information, but the difficulties in understanding this translation will deter many of the students who presumably constitute its main intended readership. Indeed it is hard to see any obvious market for this book, as water engineers and environmental consultants will continue to turn to more advanced texts. Here is an opportunity sadly missed, and a volume that is a marked contrast to some of the other, excellent titles that have been produced by this publisher. ☐

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Sea sicknesses

A. Nelson-Smith

Marine Pollution. By R.B. Clark. *Clarendon: 1986. Pp.215. Hbk £19.50, $35; pbk £9.95, $17.95.*

THERE is an enormous, widely scattered and often obscure literature on marine pollution, much of it hard to get hold of. There is, then, a need for a broad but succinct account of the subject; as editor of *Marine Pollution Bulletin* and member of several distinguished British or UN expert groups, Professor Clark is well placed to write such a book.

My review copy of *Marine Pollution* is a solidly bound paperback, only slightly larger than pocket-size and as comfortably priced. The book adequately covers the field implied by its title, with a selection of classical and contemporary examples, and opens with a consideration of the nature of pollution. Successive chapters work through the main groups of pollutant, not omitting heat, munitions, plastic waste and dredging spoil. There follows description of the state of five more-or-less enclosed seas of particular concern and, finally, discussion of the problems of assessing pollution impact.

In the interests of brevity inessential details have been omitted and explanations simplified, but the result still meets the needs of undergraduates whilst remaining comprehensible to the lay reader. The author fails to deal with a few basic points (why mention chemical oxygen demand without explaining the significance of its difference from its biological counterpart) and gives too brief an account of others (eutrophication, for example). There is, too, some unnecessary material: an account of the nature of radioactivity in less than two pages, including diagrams of atomic structure, is surely inadequate when it takes much the same amount of solid text to explain the 'critical pathway' approach when setting permitted levels for radioactive discharges. Similar inequalities of treatment are seen elsewhere —a diagram comparing three slightly different types of 'slick-licker' (used in cleaning up spilt oil) occupies most of one page, but no mention is made of the many designs of skimmer, nor of sea-booms, bubble-barriers and oil-herding chemicals that are used for containing or deflecting spills.

It is difficult for natural scientists to avoid a tone of righteous indignation when discussing pollution. Here the author succeeds admirably in maintaining the book's balance by emphasizing that marine ecosystems have a great capacity for recovery from damage; even a permanent change in component species need not necessarily be a bad thing. There is also a reminder that an improvement in effluent quality of greater than 70–80 per cent is usually impracticable, and that most pollution is in any case produced either by us or for us — a point that many armchair environmentalists conveniently ignore.

In place of a bibliography, eleven books or reports (each with extensive references) are listed as further reading. In addition, the sources of figures and tables are given as abbreviated literature citations. This, together with a comprehensive index, confirms the academic rather than popular flavour of the book, which is one I shall certainly be urging my students to read. ☐

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- *Earth*, by Anne and Paul Ehrlich, a highly illustrated "summary of Earth's environmental predicament", was published in Britain last week; the appearance of the book coincides with a three-part series, presented by the Ehrlichs, running on independent television. Publisher is Methuen, price is £14.95.

Philosophy for the nervous student

Michael Ruse

Investigating the Life Sciences: An Introduction to the Philosophy of Science. By Geert M.N. Verschuuren. *Pergamon: 1986. Pp.148. £20.95, $30.*

THIS slim volume introduces some of the basic concepts in the philosophy of science, and then develops the ideas by looking at biology — both historically and as it is today. There are four parts. The author starts with a general introduction, talking about the use (and misuse) of words, the nature of facts and whether there can ever be facts without theories, the relationship between our thought and the external world (if there be such) and so forth. Then, second, there are several brief tales about important episodes in the history of the life sciences: Harvey on the heart, Mendel on heredity, Darwin on evolution and the like.

Verschuuren then turns to some problems in contemporary biology. He is fairly conservative on the nature of science and (even more so) on its practitioners. The old distinction between the context of discovery and the context of justification is trotted out, and treated as if it were unproblematical. Nor, for instance, is the reader weighed down unduly by a major controversy going on at the moment (particularly between philosophers interested in the life sciences), about whether or not the aim in science is to find universal laws, or whether it is to find limited models which hold for one or two cases but not generally.

Finally, we get to the 'limits of science' and such issues as reductionism and determinism. My jaw dropped a little at the end, where the author states that all scientific activity since the Reformation has made little difference to our essential thinking about ethics and religion. It is difficult to believe that the fact that we are modified monkeys, contingently the end product of a long, slow, blind process of evolution, rather than a favoured miraculous Creation of a good God some 6,000 years ago, makes no difference to our religious beliefs — or to our views on right and wrong.

It seems to me that *Investigating the Life Sciences* is a book written by a teacher rather than a scholar. That sounds insufferably condescending but it is not meant in a flatly negative way. The book contains no contribution to the subject, nor (and this more of a criticism) is there any real acquaintance with much that is new in philosophy of science (in general) or philosophy of biology (in particular). But the author has a happy knack of laying out positions quickly and clearly; he does not bog down the reader with too many details — hardly possible anyway in a book of this length; and he has a real gift for setting down an idea diagrammatically, separating out concepts with a few strokes of the pen.

I doubt if I would use this book as a text on a course. But I might — and probably will — loan it to a nervous student who needs a friendly guide into the subject. □

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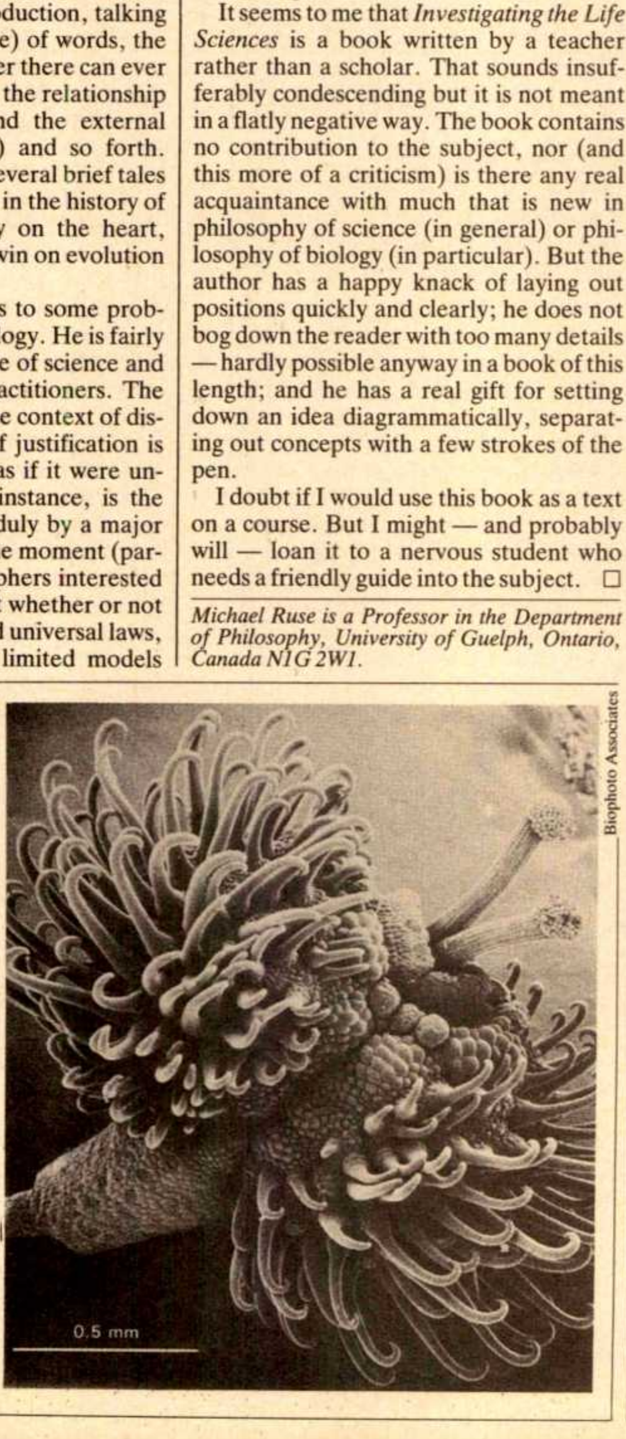

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Hitch-hiking apparatus — scanning electron micrograph of a goosegrass flower showing two developing fruits and the hooks by which, through attachment to passing animals, the fruits are dispersed. The picture is reproduced from the third edition of Karen Arms and Pamela S. Camp's Biology, *published by Saunders. The book appeared too recently to be included in the review by Cox and May on p. 225.*

Of DNA and RNA

Peter Piper

The Biochemistry of the Nucleic Acids, 10th Edn. By Roger L.P. Adams, John T. Knowler and David P. Leader. *Chapman & Hall/Methuen: 1986. Pp.526. Hbk £35, $59.95; pbk £16.95, $33.*

THIS text is now in its second generation of authors, which is the reason why editions of scientific works, unlike certain musical enterprises, do not have to end with the ninth. Previous versions of the book were, in their time, well received as concise reference sources on nucleic acid biochemistry. All of the chapters in this latest edition have been updated to the end of 1985. With its clear style of presentation, informative diagrams and extensive bibliography it will be welcomed by final-year undergraduates, and will also be used widely for reference by molecular biologists.

As the title implies, the stress is on both the structures of nucleic acids as well as the enzymes and ancillary proteins that interact with them. On the structural side, chromosomes and the ribosome are discussed in detail with an eye to their function. On the enzymological side there are lucid accounts of the metabolism of nucleic acid precursors, of DNA and RNA synthesis and maturation, of viral replication and of DNA rearrangement. Especially helpful are the various tabulations of enzyme activities, and the correlations of these activities where appropriate to the products of specific genes.

With its emphasis on the biochemistry that has led to so much of our knowledge of nucleic acids, this text is a valuable complement to other works that concentrate on the contribution made by more genetic approaches. It is generally appreciated that topics such as the enzymes involved in DNA replication, repair and recombination, the mechanisms and possible roles of RNA splicing or the actions of transcriptional control elements can no longer be given adequate treatment in those standard volumes that aim to cover the whole of biochemistry. There is therefore an increasing need for well-balanced accounts of these and similar areas, a need that this book satisfies very successfully.

This is not a text to consult for details of laboratory methods. Apparently in apology for the fact that biochemistry is an experimental subject, there is a final chapter on methods of studying nucleic acids. This is a highly readable, if succinct, introduction to the experimental side of nucleic acids and cloning. □

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Fresh transmission

Peter J. Roberts

Chemical Neurobiology: An Introduction to Neurochemistry. By H.F. Bradford. *W.H. Freeman: 1986. Pp. 507. $36.95, £21.95.*

AT THE research level neurochemistry is well established as a distinct discipline. In teaching, however, it is treated as a rather hybrid creature, various aspects being covered in courses on biochemistry, pharmacology, biology and medicine. It is therefore not surprising that hitherto there has been no integrated text on the subject at a level appropriate for undergraduates. Professor Bradford's book neatly fills the gap, and with its comprehensive listings of other relevant publications will also be of value to postgraduate students.

In his preface, the author points out the importance for neurochemists of a thorough grasp of neuroanatomy. Wisely, however, he does not attempt to provide a biochemist's introduction to the topic. An appendix provides suggestions of appropriate core texts and learning programmes, while the early part of the book describes in adequate detail the structural organization of neurons and glia, specific markers for these cell types, and their functional interactions. Energy metabolism (both *in vivo* and *in vitro*) is surveyed rather briefly, though it is fair to argue that the principles are little different from those investigated in, say, liver, and that it is justifiable to concentrate on the uniqueness of the nervous system. Non-invasive imaging techniques are also touched upon (2-deoxyglucose, PET scanning, NMR and so on), but the student is unlikely to gain much of a feel for the power and limitations of these approaches.

The emphasis of the book, as one would expect, reflects the author's interest in synaptic transmitter function. All of the main systems and their associated ionic and second messenger systems are considered, but this is not yet another book (amongst many) on transmitters — on the whole I found these sections appropriate and refreshing to read. The biochemical events underlying transmitter function are stressed, although this is put into proper context by discussing localization of pathways. The subsections on the pharmacology of the various receptor systems are rather less satisfactory. Some of this material is confusing and students, I think, would feel the urge to reach for an appropriate neuropharmacology textbook.

One of the first words that neurochemistry students learn is 'synaptosome', and Professor Bradford puts across the importance of these structures as working *in vitro* models of synaptic function with enthusiasm and authority. After discussing synaptic structure, metabolism and transmitter dynamics, he then outlines experimental uses of the preparations in relation to, for example, neurotransmitter interactions and dissection of the synaptic apparatus. The student will quickly realize what a vast amount of basic information has accrued from such reductionist approaches to the brain.

The last two chapters diverge from basic neurochemistry. One deals with central neurotransmitter systems and behaviour (behavioural pharmacology) — this area is still very much a minefield, and although certainly of interest to the student I feel that inclusion of discussion of the subject was a mistake. The other chapter contains a good description of transmitter dysfunctions in the classical neurological and neuropsychiatric disorders. This does not add much to the many other chapters in other accounts of this topic, but when viewed as part of the book as a whole it fits well and again is well referenced with up-to-date papers and reviews.

Chemical Neurobiology is uniformly well written, and there are many clear micrographs and informative figures. The production, too, is of a high standard and the layout is easy on the eye. The book is bound to be popular, and deservedly so. □

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Learned pursuits

Geoffrey Hall

Introduction to Comparative Cognition. By Herbert L. Roitblat. *W. H. Freeman:1986. Pp.377. Hbk $29.95, £29.95; pbk $17.95, £17.95.*

ONE of the main aims of the experimental study of learning in laboratory animals has been to build the foundations of a general theory for all behaviour. The rat in the maze and the pigeon in the Skinner box have been seen as model preparations from which the psychologist might conveniently derive general principles in much the same way, say, as the geneticist makes use of *Drosophila*. And just as the geneticist's laws of heredity have formed the basis of an understanding of human patterns of inheritance, so, it was hoped, studies of animal learning might help us to understand human cognition. Broadbent's seminal book *Perception and Communication* (Pergamon, 1958), the starting point for modern cognitive psychology, drew upon theories developed by students of animal learning.

Now the tables are turned. Animal-learning theorists have failed to produce a widely accepted general theory, and have watched with some envy the apparently rapid progress being made in the study of human information-processing. Some of them, and Roitblat was one of the leaders of the trend, have adopted concepts from human cognitive psychology (in particular, theories of working and reference memory, and the notion that environmental events engender mental representations that are subject to further processing) in their analysis of animal learning. Roitblat has now made the first attempt to provide a textbook, appropriate for advanced undergraduates, summarizing the new approach, and on the whole he has been successful. The material is well organized, the style is clear and every effort has been made to render intelligible the complex experimental designs that this approach to animal learning often gives rise to. The chapters reviewing studies of working memory and the sensitivity of animals to time and serial order, topics that Roitblat clearly sees as lying at the heart of his subject, are particularly useful.

A couple of doubts remain, one concerning presentation and one more fundamental. First, Roitblat presents comparative cognition as constituting a paradigm shift, a scientific revolution in which the earlier behaviourist approach is overthrown. Surely this is too large a claim. As the author is aware, many of the cognitive concepts he espouses have been employed without fuss and for some time by workers in the behaviourist tradition — indeed, one of the strengths of the book is the way in which it integrates earlier work on associative learning and conditioning with more recent studies of animal memory.

The second point concerns the relationship between cognitive processes in humans and non-humans. Many have argued that our species, perhaps by virtue of its language, possesses a set of cognitive mechanisms that other species lack. If this is so, then an attempt to account for animal cognition using theories and concepts derived largely from studying the ways in which humans process verbal material is unlikely to make much progress. Roitblat gives us a brief survey of the attempts that have been made to teach some version of a human language to apes and to a few other species, but fails to deal with the central issue directly.

Perhaps the proof of the pudding is in the eating, however: if the next few years see the fulfilment of the promise of this book — the development of a unified account of cognition in animals and man — then the doubts of the sceptics will be laid to rest. □

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M. WALLIS, University of Sussex, S.L. HOWELL, Queen Elizabeth College, London, and K.W. TAYLOR, The London Hospital Medical College

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Problems of living together

D.H. Jennings

Symbiosis: An Introduction to Biological Associations. By Vernon Ahmadjian and Surindar Paracer. *University Press of New England: 1986. Pp.212. $32.50, £26.90.*

The Biology of Symbiosis. By D.C. Smith and A.E. Douglas. *Edward Arnold: 1987. Pp.302. Pbk £16.95, $29.95.*

IN THE 1960s, the Biological Sciences Curriculum Study — that important American initiative in school biology — showed that biology could be approached starting off with the cell, the organism or the ecosystem. Today one might begin with symbiosis, the permanent or long-lasting association between two or more species. If that approach to biology were to be adopted at university level, these two books would be standard reading. In their different ways they are excellent introductions to the subject, demonstrating why investigation of the often intricate ways in which a symbiotic association may function is such a fascinating exercise and that it is open to contributions from anyone, whether they be the patient observer or the molecular biological whizz kid. And although we may here be speaking of textbooks, both can be read with profit by all professional biologists whatever their background. Photosynthesis, immunology, *r* and *K* selection and many other aspects of modern biology are all touched upon, but from the unique viewpoint of symbiosis.

Ahmadjian and Paracer are all-embracing in their approach. Rightly taking the definition above as provided by de Bary, they survey associations involving all organisms from viruses through bacteria, to unicellular eukaryotes and multicellular organisms such as helminths and mistletoes. The style is succinct, but perhaps a little breathless, and this is not a book to be read continuously. Nevertheless a student will gain much information from a chosen chapter or section. Though there are excellent half-tone figures, the diagrams leave something to be desired and a few will cause confusion among the uninitiated. But what the book lacks above all, perhaps, is a real feeling for the experimental challenge in probing a symbiotic association.

It is this particular theme that runs through Smith and Douglas's text and makes it so stimulating. Their account is more circumscribed than that of Ahmadjian and Paracer, dealing with what can be called mutualistic associations involving very intimate contact between the partners, one of which is a micro-organism, such as algae in protists, corals and lichens, mycorrhizal fungi and the rumen microflora. But the authors do marvellous justice to such matters as how we can quantify benefit or how we can identify the recognition systems involved. Numerical information is used extensively, there are good half-tones and the diagrams are models of how to convey information in an uncluttered manner. While one can grumble about the title, which is somewhat misleading, the book sets out much of the experimental evidence that underpins the symbiotic theory of the origin of the eukaryotic cell and provides valuable insights into microbial ecology as it relates to the host organism as a habitat.

Where both sets of authors fail somewhat is in their overviews at the end, in that neither grapples with the central issue of self and non-self. I suspect, however, that some other text is required to do that properly, and it is a measure of the success of these two books that they provide the foundation for a better consideration of the broader issues. ☐

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Biology writ large

Edward C. Cox and Robert M. May

Biology. By Peter H. Raven and George B. Johnson. *Times-Mirror–Mosby/Blackwell Scientific: 1986. Pp. 1,198+. $38.95, £27.50.*

Biology. By James M. Barrett, Peter Abramoff, A. Krishna Kumaran and William F. Millington. *Prentice–Hall: 1986. Pp. 1,167. $42.65.*

Biological Science, 4th Edn. By William T. Keeton and James L. Gould. *W.W. Norton: 1986. Pp. 1,175. Hbk $36.95; pbk £19.95.*

The World of Biology. By Thomas G. Overmire. *Wiley: 1986. Pp. 557. $29.25, £28.30.*

THE competition among introductory biology texts over the past ten years and more provides an off-beat example of what evolutionary biologists call Cope's Law — a tendency to ever-increasing body size. Indeed, conventional bibliographical details in book reviews omit what to many students may come to be the most important parameter characterizing these books: Raven and Johnson weighs 3.4 kg, Keeton and Gould 2.8 kg and Barrett *et al.* 2.9 kg. It may be time for publishers to start thinking of issuing textbooks as a collection of separate volumes, or else to bind them in carrying straps or even on wheels.

This trend towards giantism derives from our growing understanding of the biological world, at every level from molecules to ecosystems. Publishers and authors presumably believe — probably correctly — that this expanding body of knowledge has to be surveyed fully, lest a competing text be seen as more comprehensive or lest a lecturer's own interests be omitted. It would be nice to see an attempt to present introductory biology in a more digestible way that focused on a few central themes (as distinct from striving after relative brevity by reducing the ocean of facts to a vast, shallow lagoon of them). Such a text would, however, probably be seen as idiosyncratic.

Another conspicuous tendency is the increasing excellence of the illustrations in introductory biology texts, as competitors strive to match the high standards originally set by Curtis. It makes the present books a pleasure to thumb through, although there is a danger that pedagogic purpose may occasionally be subordinated to illumination of the page.

Barrett *et al.*, and Raven and Johnson, are both new books. Raven and Johnson, indeed, explain at the outset that their book is based on extensive intellectual and marketing surveys, and they scold other authors for perpetuating inaccuracies and for cribbing from each other. The organization of the book is interestingly different from the usual molecules-through-cells-to-organisms: it begins with sections on the origins of living things, the biology of the cell, genetics and heredity, and evolution; it then moves on to the biology of simple organisms, of plants, of invertebrate animals and of vertebrates, and concludes with ecology. This reorganization works well. Such fundamental rethinking has had some concomitant 'oopses'. Immunology, for example, is left out entirely; a supplementary chapter on the subject is now available, and will be bound into later editions. And there are several errors, although these are corrected in a later list of errata which has been mailed out. For instance, those familiar with the history of ideas about altruism and kin selection will be interested to read an account of "a passing remark" by Haldane (no mention of Hamilton anywhere), to the effect that he would lay down his life for two brothers or four first cousins. This error (it should be eight first cousins) is reiterated in a problem, to which the wrong answer is given ("would you lay down your life for one brother and three first cousins?"). Not surprisingly — Raven is a distinguished ecologist — the ecology section is good, and an evolutionary perspective suffuses the text in a way we like very much.

The book by Barrett *et al.* is organized along more conventional lines, little-to-big. It begins with a thoughtful discussion of what science can and cannot be, what its rules are, why it is not — and perhaps never can be — complete, and how it can benefit society yet why that cannot be the only measure of success. This book is better written than most, with the right pace, a clear and direct style, good use of language, and excellent connections between text and illustrations. First-rate essays (set as what the authors call "panels") enliven the accurate text. Like most introductory biology texts, however, this book lacks the pervasive evolutionary perspective of Raven and Johnson.

Jim Gould has produced a thorough revision of Keeton, which (with Curtis) has led the field in recent years. Gould is a colleague of ours, and conflict of interests forbids us from more than briefly mentioning this book. We would, however, urge teachers of introductory biology to read it, and to examine the comprehensive set of accompanying material (laboratory guide, study guide, teacher's manual, sample tests).

Overmire's *The World of Biology* is a shorter and simpler book, aiming to contend with the reduced versions of Arms and Camp or Keeton for adoption in more elementary survey courses. It is conventionally organized, accurate and 'simply written' in a way that, although clear, makes minimal assumptions about the vocabulary of the reader. Overmire manages to convey a real sense of concern for current environmental problems, without forcing opinions down the reader's throat. The illustrations are good, and well integrated with the text, although not up to the extraordinary quality of the three big books. ☐

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Life's processes

Ole H. Petersen

Physiology and the Scientific Method: The Design of Experiments in Physiology. By Evelyn M. Scott and James M. Waterhouse. *Manchester University Press/Longwood, Wolfeboro, New Hampshire: 1986. Pp.204. Hbk £20, $34.50; pbk £8, $14.*

Principles of Physiological Measurement. By James N. Cameron. *Academic: 1986. Pp.278. Hbk £49.50, $59.50; pbk £20, $23.95.*

Lecture Notes on Human Physiology. Edited by John J. Bray, Patricia A. Cragg, Anthony D.C. Macknight, Roland G. Mills and Douglas W. Taylor. *Blackwell Scientific: 1986. Pp.616. Pbk £9.95, $19.50.*

IS THERE anything useful to be said about *physiology* and the scientific method? Having read Scott and Waterhouse's book with this title one is tempted to give the answer 'no' and to recall Popper's statement that "We are not students of some subject matter but students of problems". The less ambitious subtitle is *The Design of Experiments in Physiology*, and the book is in fact intended as a practical guide to experimental work for final-year physiology students.

The first part deals with such matters as choosing species and types of preparation, experimental design, healthiness of preparations, measurements and recordings, presentation and statistical assessment of results, and finally with drawing conclusions. The second part consists of six 'abstracts' based loosely on published papers. Each of these abstracts is a very brief version of a scientific article followed by a number of questions and suggested answers, all intended to illustrate some of the general principles set out in the first part of the book. The authors are very keen on the 'physiological normality' of experimental preparations, but seem unaware that a great many important experimental results have been obtained, and could only be obtained, under conditions that are very far from normal. In all, the book is superficial, and because of the arbitrary selection of material will not meet the needs of most students.

Another 'general principles' book, by James Cameron, deals with physiological measurements. The aim has been to provide a "compact reference for the commoner methods in use in physiology as an entry point for the student without such exposure". In a short volume such as this the main problem is the selection of material. Overall, gas analysis, which seems to be the main interest of the authors, dominates the contents. But although this is an important topic, it does not justify the general title of the book. There are also chapters on radioactive tracers and some basic ones on gas and solution concepts, electronics and transducers, but, astonishingly, none on electrophysiology. It is very hard to think of a physiology course that contains no neurophysiology, cardiac electrophysiology or membrane physiology; indeed, these topics would dominate many courses, and the fact that this book has nothing to say about extra- or intracellular electrical recordings, not to mention the increasingly important area of single-channel current recordings, is a major defect.

This is a pity, for some of the sections, for example that on pipetting, are excellent with many helpful practical details that are difficult to find elsewhere. In the chapter on *p*H measurements, however, important advice concerning calibration is missing. Many laboratories are unaware of errors arising from liquid junction potentials when *p*H meters with the commonly used combination electrode are employed. The point should have been made that *p*H calibration needs to be done using salt concentrations relevant to the test conditions in order to avoid errors which can be as large as half a *p*H unit.

The modestly titled *Lecture Notes on Human Physiology*, edited by a group at the University of Otago Medical School, is a short but comprehensive textbook of physiology, well suited to most courses for medical students. Of the many shorter physiology texts that I have reviewed in recent years this is by far the best. It is written with great clarity, using simple but precise diagrams of uniform style, and contains virtually no important errors. It is a pleasure, for once, to see a correct description of the relationship between aortic and left ventricular pressures, and to have good explanations of both cardiovascular and respiratory changes in the transition from rest to exercise.

Equally impressive is the chapter on kidney, water and electrolytes, which is clear and up to date, and contains more precise information than is found in many larger textbooks. Having previously complained about the lack of discussion of osmotic diuresis in several other texts, I am glad to see that this point is well explained here. Inevitably there are some weaknesses, and the chapters on endocrinology and gastrointestinal function stand out as being less complete and up to date than the others.

It often takes a long time for new concepts to be incorporated into textbooks, and in physiology the situation seems to be worse than in many other subjects. In *Lecture Notes on Human Physiology* it is disappointing to find that although the patch-clamp method for single-channel current recording is introduced with a schematic diagram, virtually no use is made of single-channel information in the discussions of electrical activity in nerve, muscle and other cells. This is a mistake, because a molecular approach to membrane physiology makes it much easier for students to understand basic ideas in this field. Now that physiology has its own short review journal, *News in Physiological Sciences*, we must hope that textbook authors will find it easier to keep up with developments and will take the trouble to integrate novel concepts into the main text when these serve to clarify the fundamentals of the subject. ☐

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- Also published last year, by Raven, was a second edition of *Respiratory Physiology* by Allan H. Mines. The book has been up-dated generally, and new topics included, and like its predecessor is intended mainly as a course textbook for students of medicine and allied subjects. Price is pbk $19.50.

Going through the motions

Judith Armitage

Cell Movement and Cell Behaviour. By J. M. Lackie. *Allen & Unwin: 1986. Pp. 316. Hbk £30, $45; pbk £11.95, $19.95.*

As ITS title declares, this book undertakes to cover all of cell movement and its control, from muscle to amoeba, from mitosis to bacterial flagellar rotation. The author admits in his preface that no one person can know closely more than a small part of this now-huge field of research, so from the outset he set himself a very difficult task. I understand, however, that there was also an inordinate delay between the submission of the typescript to the publisher and its appearance in printed form. The result is a book which might have been exceptional, but as it is can only be recommended with reservations.

Dr Lackie is an expert in leukocyte movement, and in dealing with this subject his references are up to date and the writing flows with ease. This readable style overflows into the accounts of other areas, for example those on bacterial motility and chemotaxis. Here, however, arguments are put forward for cyclic GMP being the sensory signal, an idea now all but abandoned; also gene names are used and gene product roles are assigned that have been out of date for several years. Similarly, it is unfortunate to see a chapter in a book published in August 1986 beginning with the statement that "The association of microtubules with movement is, with one important exception, rather more an article of faith than a well documented reality..." — new techniques turned that faith into reality some time ago. These points may seem to be not particularly important, but misinformation of this sort will confuse the advanced students taking courses on immunology, pathology and cell biology at whom this book is aimed.

To be fair, the sections I have criticized are a small part of the book as a whole. Because of Dr Lackie's imaginative approach, the rest of it will provide students with an illuminating view of the subject. There are three main sections. They deal with different types of motor; with how these are used by different cells to move, including clear explanations of why each is suited to a specific task; and with the differences in the use and control of these mechanisms in uniform and anisotropic environments. Particularly good are the introductions at the beginning of each section, where Dr Lackie explains by analogy and with simple diagrams the problems of size, shape, surface and gradient faced by single cells, and really gets the reader to think down to the size of the individual cell.

The book is illustrated with well-planned diagrams to support the text and with plenty of electron and light micrographs. Again, however, in the accounts of bacterial motility and microtubules many of the figures should have been updated.

Overall this is a highly readable and digestible book which will be very useful to students of eukaryotic cell biology. But anyone reading it should take the bacterial and microtubule sections as historical rather than providing a current view of the field. ☐

Judith Armitage is a Lecturer in the Microbiology Unit of the Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK.

Sole contributions

John Edwards

The World of the Cell. By Wayne M. Becker. *Benjamin/Cummings:1986. Pp. 882. $39.95, £36.95.*

Molecular Cell Biology. By Charlotte J. Avers. *Addison-Wesley:1986. Pp.704. $19.95, £19.95.*

Genetics & Molecular Biology. By Robert Schleif. *Addison-Wesley:1986. Pp.626. $20.95, £20.95.*

Molecular Biology, 2nd Edn. By David Freifelder. *Jones & Bartlett:1987. Pp.834. $38.75.*

THE ever-expanding synthesis of cell and molecular biology has been celebrated in two outstanding multi-author textbooks: *Molecular Biology of the Cell* by Alberts *et al.* and, more recently, *Molecular Cell Biology* by Darnell *et al.*

Single authors of new texts will argue that for some levels or periods of study these volumes on the one hand may overwhelm with their sheer scale or, on the other, sacrifice depth for breadth. But those who set out single-handed to tackle the broad canvas can know only a small part of the relevant primary literature. This may not matter too much for presenting neat accounts of the latest models, but the how and why inevitably suffer.

Another result can be the propagation, particularly among peripheral material, of convenient oversimplifications, curious choices of example or even downright myths. For the first, see the description of Frye and Edidin's antigen-mixing experiment in the books by Avers and Becker (both label and fuse in the wrong order). For the second, the Alberts-like inclusion of a sponge aggregation factor and omission of the well-characterized vertebrate cell adhesion molecules in Avers. For a myth, that transformed cells in culture have lost contact inhibition of locomotion (Avers) or, worse, that this loss *causes* them to grow to higher densities (Becker).

Becker's sweep is broad, "a comprehensive introduction", from biochemistry (with the emphasis on energy), through organelles and molecular genetics, to development, motility, the nervous and immune systems, and cancer. Of the problems that follow each chapter, some simply check if the reader stayed awake. Others are imaginative and entertaining, and together with some jolly analogies and an occasionally breezy style show an author working hard to make the material vivid for students.

I am not convinced, however, that Becker succeeds very well in his declared aim "to explain not only what we know, but also how". For example, the account of muscle contraction features the cross-bridge cycle, but apart from a fragment of evidence in the problems the only experimental source hinted at is electron microscopy. Here and elsewhere I would have preferred to see a few more comments such as "it is thought that" or "one idea is", rather than the stone-tablet style. Despite trumpeting for the cytoskeleton, microfilament bundles and tissue cell locomotion are omitted. The material on tumour cells is weak. I fear the experiment on cell sorting is imaginary. The choice of images of fluorescent microtubules to illustrate effects of transformation on the cytoskeleton is unfortunate, because leading practitioners are on the record as stating that this effect is an optical artefact. And a curious omission from the essay on *ras* is mention of the point that p21 has hydrolytic activity towards the GTP it binds, and that this activity is decreased by the oncogenic mutations. For a book at this level I prefer Karp's *Cell Biology*, reviewed here in 1985.

Charlotte Avers's *Molecular Cell Biology* is perhaps one-third the length of the multi-author work of the same name. The subset of material she has chosen has the intact cell as the upper limit rather than the centre, and the emphasis is strongly eukaryotic. Chapters on cell surfaces, organelles and genome are followed by "Reproduction and Development" (the cell cycle, mitosis and meiosis, rather than a foray into embryology), and cellular and molecular evolution. There are flaws. In the introductory biochemistry, should not the idea of structures being favoured by hydrophobic clustering appear in relation to protein conformation as well as membranes? In two figures, hyperbolic curves are shown reaching their asymptotes when they should still have some way to go. Later, decamin is said to be a different intermediate filament protein of fibroblasts, but it was in fact an earlier name given to the pair now usually known as vimentin and desmin.

The core of the work, however, is

admirably done. Here Avers manages concisely and thoughtfully to indicate historical perspectives, some of the key experimental evidence and, most importantly, the existence of areas of ignorance and of more than one point of view on a number of issues.

I had not met David Freifelder's *Molecular Biology* in its first edition, but have enjoyed dipping into the second. This is very much molecular biology in its traditional territory of DNA makes RNA makes protein, although the book does include a good deal of the newer eukaryote material. A glance at almost any topic shows how this gutsy discipline gets watered down in texts which try to assimilate it with the whole of the new biology. I wonder if this particular subset of the information will continue to be sustainable, however, as the tools of the trade are applied increasingly to supracellular problems such as development. A hint of future difficulties can be felt in Freifelder's need to sketch in matters cell biological in order to discuss the action of oncogenes.

Schleif's *Genetics & Molecular Biology* covers much of the same ground, but otherwise is a world apart, and a remarkable achievement. It is an advanced text, derived from material taught largely to postgraduates, and will probably be thought best suited to budding professionals in molecular genetics. In some ways this would be a pity, because there is also gold here for the rest of us. One feature is the prominence given to thinking about the engineering logic of the systems under consideration. For example, why are great complexities engendered by discontinous replication of DNA on the lagging strand when cells could perfectly well have evolved a polymerase working continuously 3′ to 5′? How do bacteria survive the probable error rates of their own restriction enzymes? The answers are perhaps given rather dogmatically, but other authors do not pose such questions let alone open discussion of subjects with them.

A second feature of Schleif's book is the constant encouragement of calculations, both in the text and in the challenging problems, reminding us that molecular biologists should still keep a stock of old envelopes as well as restriction enzymes. "Deeper Reading" lists which follow each chapter mean just that — in some areas they appear to cover an appreciable proportion of the primary literature. The lessons here in dealing with the information explosion in biology are that an ounce of rationale is worth a pound of facts and that, for educational value, there is nothing to beat an author writing about stuff he knows from the inside. ☐

John Edwards is a Senior Lecturer in the Department of Cell Biology, University of Glasgow, Glasgow G12 8QQ, UK.

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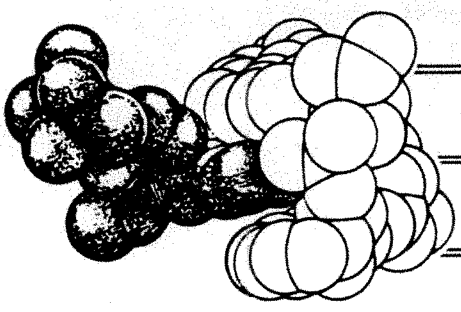

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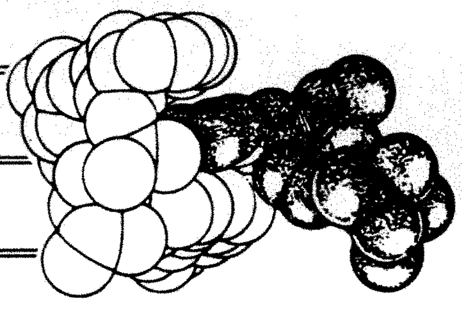

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Applications, accompanied with curriculum vitae, should be addressed to the Director of the Research Centre, Dr R. Singh Grewal. (W3889)A

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Boehringer Mannheim is a rapidly growing leader in the field of medical diagnostics and biochemicals. This leadership has been attained through emphasis on quality and team effort and it's Boehringer Mannheim's employees that are responsible for growth and leadership. Due to this growth, we are currently seeking qualified individuals for exceptional opportunities in the following areas:

RESEARCH INVESTIGATOR — TEST DEVELOPMENT

Will coordinate and conduct research and development of clinical chemistry diagnostic tests in novel formats from initial laboratory experiments through scale-up and evaluation to transfer into production. Requirements include a PhD or equivalent experience in Biochemistry, Chemistry or related area and proven research and development experience in test development preferably in an industrial setting. Effective oral and written communication skills and supervisory skills are also essential.

RESEARCH INVESTIGATOR — ANALYTICAL

Responsibilities include developing specifications and analytical procedures for characterization of raw materials and finished products with regard to their composition, reactivity, enzyme activities and stability. Also will coordinate and develop in process quality control procedures. A PhD in Analytical Chemistry, Biochemistry or equivalent and knowledge of a wide variety of analytical instrumentation is required. Effective oral and written communication skills and supervisory skills are also essential. Expertise with polymers would be a definite plus.

RESEARCH INVESTIGATOR — TECHNOLOGY DEVELOPMENT

Will conduct research in new technologies directed toward test development in novel formats. Will also provide an interface between test development scientists and engineers to develop instruments and equipment to bring new technologies on line. A PhD in Chemistry, Biochemistry, Physics or an MS in Chemical Engineering or equivalent experience is required. Effective oral and written communication skills are also essential. Experience with film technology is a definite plus.

RESEARCH INVESTIGATOR — IMMUNOASSAY DEVELOPMENT

Will coordinate and conduct research and development of immunodiagnostic tests in novel formats from initial laboratory experiments through scale-up and evaluation to transfer into production. Requirements include a PhD or equivalent experience in Biochemistry, Chemistry or related area and proven research and development experience in test development, preferably in an industrial setting. Effective oral and written communication skills and supervisory skills are also essential.

SCIENTISTS

These positions will support the above personnel with requirements including B.S. or M.S. in Biology, Chemistry, Biochemistry, Immunochemistry or Analytical Chemistry and at least one year of applicable R&D experience.

At Boehringer Mannheim we offer an exciting and challenging work environment as well as an excellent compensation and benefit program including a 401K. If you meet the above requirements and would like to join an organization that is a leader in its field, we invite you to send your resume with salary history to:

HUMAN RESOURCES
BOEHRINGER MANNHEIM
Department N • P.O. Box 50100
9115 Hague Road • Indianapolis, IN 46250

(NW632)A

BOEHRINGER
MANNHEIM
DIAGNOSTICS

An Equal Opportunity Employer

The Future Looks Excellent

Anheuser-Busch is planning today for the 21st century. New ideas are being explored. New people are being hired, trained and groomed for increased responsibility. New areas of business are being researched and developed. Look to tomorrow with Anheuser-Busch, and to career excellence in the following position:

Biotechnologist

In this position, you must possess a Ph.D with emphasis in plant biotechnology; familiarity with micro-computers; a background in molecular biology and plant-related biotechnology research (especially agricultural related); the ability to assimilate, synthesize and oversee biotech research directed at agricultural problems. You must also have knowledge of immunological techniques — antibody production, purification and utilization; excellent communication and presentation skills; and practical experience in clonal propagation, callus regeneration, protoplast regeneration, soma-clonal variation and selection genetics, cytology, and any of the following genetic engineering techniques — cloning, hybridization and restriction mapping.

At Anheuser-Busch, you can earn an excellent salary and comprehensive benefits. Please send your resume today to: **ANHEUSER-BUSCH COMPANIES; Corporate Employment; Department JM-DD-N; One Busch Place; St. Louis, MO 63118.** Equal Opportunity Employer M/F.

(NW606)A

ANHEUSER-BUSCH
COMPANIES

PLANT PATHOLOGIST

Research Team Leader

ICI Plant Protection Division – the fastest growing ICI Division – is at the forefront of the vital growth area of worldwide crop protection. Our rapid growth and commitment to continued discovery and development of new products, in our excellent research and testing facilities, creates many exciting and challenging career opportunities.

We are currently seeking a recently qualified PhD Plant Pathologist with a good organic chemistry/biochemistry background and managerial skills to lead the primary screening team in Plant Pathology section. Your aim will be to discover potential new agricultural fungicides by elucidating structure activity relationships and devising new screening methods. You will require a knowledge of the major fungicide classes and of their use on the major food crops.

We offer a highly competitive salary supported by excellent company benefits including relocation expenses where appropriate.

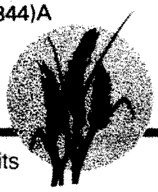
Please apply in writing enclosing full c.v. quoting Ref. TO/PP to: Joe Booth, Personnel Officer, ICI Plant Protection Division, Jealott's Hill Research Station, Bracknell, Berks RG12 6EY.

Closing date for applications is 26th March 1987.

ICI is an equal opportunities employer

(2344)A

ICI
Plant
Protection
Division



The world harvests the benefits

NATIONAL
CANCER
INSTITUTE

FREDERICK CANCER
RESEARCH FACILITY

P.O. Box B, Frederick, Maryland 21701

NATIONAL CANCER INSTITUTE — FREDERICK CANCER RESEARCH FACILITY BIONETICS RESEARCH, INC. — BASIC RESEARCH PROGRAM

MACROMOLECULAR CRYSTALLOGRAPHERS

The Basic Research Program is presently recruiting scientific staff for a new crystallography laboratory headed by Dr Alexander Wlodawer. The laboratory will study the structure and function of proteins and nucleic acids and is equipped with state-of-the-art data collection and processing tools, including an X-ray detector and molecular graphics. The Computing Laboratory at the Frederick Cancer Research Facility is equipped with a Cray X-MP supercomputer and two large VAX computers.

Applications for the following positions are now being accepted:

Section Head or Group Leader — scientist of recognized merit with leadership ability and at least 5 years of relevant postdoctoral experience, include a research plan with application.

Scientist Associates — scientists with demonstrated ability for independent work with at least 2 years of postdoctoral experience.

Scientific Programmer — knowledge of Fortran and experience operating a VAX/VMS system. Experience with the Cray computer also useful.

Research Technician — experience in protein purification and characterization desirable but not required.

Postdoctoral Fellow(s) — recent PhDs in protein crystallography or other aspects of structural biology.

To apply, send curriculum vitae and the names of three references to: **Dr Maurice Guss, Executive Officer, BRI-Basic Research Program, NCI-FCRF, P.O. Box B, Frederick, MD 21701**. An equal opportunity/affirmative action employer. M/F/H/V. (NW618)A

BIONETICS RESEARCH • BASIC RESEARCH PROGRAM

RESEARCH OPPORTUNITIES IN BASIN STUDIES

ANTICIPATED RESEARCH ASSISTANT PROFESSOR IN BASIN STUDIES: The LSU Basin Research Institute seeks a highly qualified PhD to participate in integrated multi-disciplinary basin studies with emphasis on the subsurface. Preference will be given to candidates with significant seismic stratigraphic and sedimentologic experience (both carbonate and clastics) in the Gulf coast province. Industrial experience and a publication record are highly desirable. Joint appointment with the Department of Geology, or the Louisiana Geological Survey may be considered.

POSTDOCTORAL FELLOW IN CARBONATE STUDIES: Applied Carbonate Research Program of the LSU Basin Research Institute anticipates the availability of a postdoctoral research position for the academic year '87-88. Research opportunities include: Carbonate Petrology, Geochemistry, and Stable Isotope studies. Research emphasis will be on Diagenesis.

POSTDOCTORAL FELLOW IN SILICICLASTIC DIAGENESIS: LSU Basin Research Institute anticipates the availability of a postdoctoral research position for the academic year '87-88. Research program is flexible, but preference will be given to candidates with experience in the combined areas of Petrography and Geochemistry.

Interested candidates should send application letter, resume and list of references to: **Dr Clyde H. Moore, Director, Basin Research Institute, Louisiana State University, Baton Rouge, LA 70803.**

LSU is an equal opportunity, affirmative action employer.

(NW627)A

POSTDOCTORAL BIOCHEMIST

A postdoctoral position is available for 3 years in the Department of Biochemistry and Physiology of the Hannah Research Institute (University of Glasgow). The successful applicant will join a small team investigating the regulation of hepatic carnitine palmitoyltransferase at the translational and post-translational levels. Applicants should possess, or expect to obtain shortly a PhD in Biochemistry or related subject. Salary will be on the first point of the Higher Scientific Officer Scale (£8,405), rising to £9,285. Applications, including full c.v. and names of two referees, should be sent to the **Secretary, Hannah Research Institute, Ayr KA6 5HL.**

Informal enquiries may be made to Dr V.A. Zammit (Tel. 0292-76013 Extn 312). (2321)A

MICROBIOLOGIST

The Department of Microbiology, University of Alberta invites applications for an independent research position from qualified individuals with demonstrated research abilities in basic or applied problems in microbiology. Candidates using biochemical/molecular approaches will be accorded special preference. Candidates should have a recent PhD and relevant postdoctoral experience. The position is a 5-year renewable term appointment at the Assistant Professor level (Salary range: $31,612—$45,340) and will be contingent upon a successful application for a competitive award from an external agency. Successful applicants pursuing research of medical relevance may also be eligible for substantial research establishment funding. Duties include development of an independent research programme, graduate student supervision and some appropriate classroom teaching. Applications, containing a curriculum vitae, names, addresses and telephone numbers of 3 or more referees, and a concise but specific description of research interests and proposed work should be sent to: **Dr S. E. Jensen, Department of Microbiology, University of Alberta, Edmonton, Alberta, Canada, T6G 2E9.** The closing date for applications is June 30, 1987.

The University of Alberta is an equal opportunity employer, but priority will be given to Canadian citizens and permanent residents of Canada. (NW630)A

UNIVERSITY OF PITTSBURGH
DEPARTMENT OF BIOLOGICAL SCIENCES
CHAIR

Applications and nominations are invited for the position of Chair of the Department of Biological Sciences at the University of Pittsburgh. Candidates should have a distinguished research program as well as the energy and ability to lead the Department into its next phase of development. Currently there are 30 tenure-stream faculty members. Additional appointments have been approved.

Within the Department, or available to its members, are facilities for DNA synthesis, protein sequencing, cell culture and virology, hybridoma production, large scale fermentation, and electron microscopy. The Department trains 60 graduate students in addition to 350 undergraduate majors. Candidates from all areas of modern biological research are welcome. Review of applications will begin April 15, 1987. Applicants should send a curriculum vitae and the names of potential referees to:

James M. Pipas, Chair Search Committee, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

The University of Pittsburgh is an EEO/AA Employer.

(NW614)A

POSTDOCTORAL POSITION

now available in biochemistry. We are an established group in the area of membrane structure and function, magnetic resonance spectroscopy and biophysics, with interests in signal transduction, protein kinases, phospholipase c, and molecular basis of tumor promotion. We are looking for a colleague with mutual interests and a strong background in biochemistry. Cloning experience helpful. Send vitae and names and addresses of three references to: **Dr O. Hayes Griffith, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403** by April 15, 1987. Telephone: (503) 686-4634. *An Equal Opportunity/Affirmative Action Employer.*

(NW607)A

MOLECULAR AND CELLULAR ASPECTS OF REGULATORY BIOLOGY

The Banting and Best Department of Medical Research, University of Toronto, is seeking candidates for the position of Assistant Professor (Tenure stream). The position is available July 1, 1987 and will carry an initial appointment of five years at a competitive salary. Candidates should have a strong research background and be prepared to develop a research program in Molecular and Cellular aspects of Regulatory Biology (for example - Molecular Endocrinology, Mechanisms of Signal Transduction, Regulation of Gene Expression). The successful candidate will be expected to attract independent research grants, to spend at least 75 per cent of his/her time in research and to participate in graduate and undergraduate training.

Applicants should forward a curriculum vitae, an outline of research interests and three letters of reference to: Dr David H. MacLennan, Professor and Chairman, Banting and Best Department of Medical Research, University of Toronto, 112 College Street, Toronto, Ontario, Canada M5G 1L6. In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada, but foreign applications are encouraged.

(NW577)A

ASSOCIATE R & D DIRECTOR
CSL
AUSTRALIA

CSL is Australia's leading manufacturer of biologicals and has a strong commitment to Research and Development. CSL's major facilities are located at Parkville which is four kilometres from the centre of Melbourne, and is in close proximity to the University of Melbourne, the Walter and Eliza Hall Institute of Medical Research and several other institutions.

CSL is looking for a senior researcher with the ability to manage, plan complex R&D activities, develop and implement strategies compatible with our corporate goals, adapt to changing technological needs, and motivate and direct a team of researchers. The right person will make significant contributions to the identification of new business opportunities, translate the results of scientific programs into recommendations for corporate action, and make sure projects operate efficiently, effectively and economically.

This new position will be one of two Associate R&D Directors who will each share responsibility for the R&D Group and manage their respective areas of activities and projects.

Required experience includes a tertiary qualification in medicine, veterinary science or a higher degree in one of the biological sciences and academic and/or industrial research experience and possess ability or potential as a senior research manager.

We offer a competitive salary, removal expenses and associated benefits for this appointment which we would expect to make about mid-1987.

Please send a c.v. outlining your qualifications, description of scientific and research experience and the names of two referees, to: **Mr E.D. Eade, Personnel Director, Commonwealth Serum Laboratories, 45 Poplar Road, Parkville, Vic. 3052 Australia, by 23 April, 1987. Telephone enquiries may be made to Dr Peter Schiff, R&D Director, 613-3891484.**

(W3909)A

CSL
COMMONWEALTH
SERUM
LABORATORIES

AUSTRALIA

The University of New England

Equal Opportunity is University Policy

DEPARTMENT OF ECOSYSTEM MANAGEMENT

LECTURER IN ECOSYSTEM MANAGEMENT

Vacancy is in the field of Land Resources Management (e.g. of rangelands, forests, arid lands, coastal zones, conserved lands).

The department teaches courses in the science and management of park, wetland and wildlife resources and land use, as part of the undergraduate degree, Bachelor of Natural Resources. It has strong research programs in these areas and the underlying biological and environmental disciplines.

Applicants should have basic training in one of the biological disciplines underlying ecosystem management. Subsequent involvement in ecosystem analysis, resource management, policy and planning, applied ecology or a related area is essential, as is a PhD or equivalent. The appointee will have demonstrated research potential preferably in the form of published work.

The candidate will teach in courses within the department appropriate to their training and background. Participation in courses on management and/or policy would be particularly encouraged. They will also be expected to mount a research effort and supervise postgraduate students at all levels.

Further details from **Professor R. L. Kitching on 61-67-73 2539.**

Salary: A$27,859 — A$36,600 per annum
Closing Date: April 22, 1987
Position No.: 433

The appointment will be to the permanent staff but the University reserves the right to make the appointment probationary where it considers this appropriate.

Other conditions include superannuation, assistance with travel and removal expenses and with buying or building a house in Armidale.

Applications, including the names and addresses of three referees and stating the position number, should be sent to the **Staff Officer, University of New England, Armidale N.S.W. 2351, Australia** prior to the closing date. Applicants should forward a copy of this advertisement to their referees and ask them to send their reports direct to the **Staff Officer, Mr. B. G. Turner,** marking their envelopes **'Private and Confidential'** and quoting the position number, before the closing date so as to expedite the appointment process.

ARMIDALE NSW 2351
AUSTRALIA (W3905)A

FACULTY POSITION

(non-tenure accruing)

DEPARTMENTS OF OBSTETRICS AND GYNECOLOGY AND BIOCHEMISTRY

A non-tenure accruing position as Research Instructor to teach and supervise graduate students and research technicians in the department of Obstetrics and Gynecology is available. The appointee would be required to design and conduct experiments to investigate the molecular mechanisms involved in the developmental and hormonal regulation of surfactant synthesis in fetal lung tissue. A PhD in Biochemistry or a related field is required and four years of postdoctoral experience in molecular biology, hormone action, preparation of cDNA libraries, the isolation of cloned cDNAs and genes, DNA sequencing, *in vitro* transcription assays, and protein purification. Submit curriculum vitae and brief summaries of past research experience with the names of 3 references to: **Associate Director, Cecil H. and Ida Green Center for Reproductive Biology Sciences, University of Texas Health Science Center at Dallas, 5323 Harry Hines Blvd, Dallas, Texas 75235-9051.** *Equal Opportunity Employer.*

(NW628)A

PHARMACOLOGIST

wanted to initiate Research Programme in Ireland

A Pharmacologist is required to established a research programme for the discovery and development of novel drugs for the treatment of neurological and psychiatric diseases. The applicant should have an interest in studying the effects of drugs on memory and learning.

This is a new venture by an Irish based Pharmaceutical Company and the position is therefore aimed at a highly motivated research scientist with several years of postdoctoral experience in the pharmaceutical industry and a proven record in drug research. Initially the research programme will be undertaken in conjunction with the Department of Pharmacology, University College, Galway.

A full curriculum vitae, stating experience, details of research publications and the names of three referees should be sent in confidence to: **Prof. B. E. Leonard, Professor of Pharmacology, Department of Pharmacology, University College, Galway. Tel: (091) 24411 Ext. 246.** (2325)A

MEDICAL RESEARCH COUNCIL

Clinical Oncology and
Radiotherapeutics Unit,
Cambridge

CAREER NON-CLINICAL SCIENTIST CELL BIOLOGIST

Applications are invited for a career non-clinical scientist post with the Medical Research Council. The post is tenable at this unit in the first instance. The successful applicant will be expected to carry out studies at the cellular and molecular level to identify and characterise tumour cell phenotypic markers for clinical application. With particular emphasis on the development of molecular probes for therapeutic responsiveness. The post will be an integral part of the unit's research programme and will complement the studies of other groups in the unit concerned with drug resistance and metabolism, DNA damage/repair, hyperthermia, oncogene
expression and novel application
of flow cytometry. The successfu
applicant will have a backgroun
in cellular biology with expertise
the production and clinical appl
cation of monoclonal antibodi
and will have demonstrated th
ability to lead independent re
search projects and initiate scien
tific collaborations.

Salary will be at the appropriat
point on the scale for non-clinic
scientific staff. Applications (
copies) including a full curriculu
vitae, with a list of publicatio
and the names of 3 referees shoul
be lodged by 26 March 1987 wit
the Director, Professor N
Bleehen, MRC Clinical Oncolog
and Radiotherapeutics Uni
MRC Centre, Hills Road, Cam
bridge, CB2 2QH from who
further details may also b
obtained. Applicants should incl
de a statement of about 1,0
words in length explaining the r
search programme that they wou
wish to carry out if appointed.

The MRC is an equal opportu
ity employer. (2353)A

SCIENCE DIRECTOR

CELL BIOLOGY

Direct research in the cell biology laboratory. Implement research techniques for development of new crop varieties through tissue culture techniques, including mutant isolation, cell fusion with emphasis on modification of cytoplasm. Develop new germ plasm for crop plant breeding, and facilitate commercialization, etc. PhD or equivalent degree in Biological Science or Genetics or Microbiology. Requires in-depth project or research background in plant cellular genetics, including isolation of cytoplasmic and nuclear plant cell mutants, and the transfer by cell fusion of chloroplast and mitochondrial genetic traits. Research must also include selection, isolation and characterization of chloroplast-encoded mutations in plant cell cultures. Must also have record of publication in major scientific journals. $5,916/mo; 40 hrs./wk. Place of employment and interview: San Francisco/Bay Area, CA. If offered employment, must have legal right to work. Clip ad and send with resume to: **Job # CW1396, P.O. Box 9560, Sacramento, CA 95823-0560,** not later than March 30, 1987.

(NW623)A

ANALYTICAL CHEMIST

in Stability Testing Programs, in the area of finished pharmaceutical dosage forms, inclusive of solids, liquids and aerosols. Job responsibilities include performing all physio-chemical tests required in stability protocols, assisting in method development, including the use of robotics, and initiating repair of analytical instrumentation. Minimum requirements include a Master of Science in Industrial Chemistry, together with three years industrial pharmaceutical experience in the testing of raw materials, and finished dosage forms, including stability testing according to compendial and/or Company procedures, including time release formulations. Also required is demonstrated expertise with HPLC, TLC, GC, IR, automated instrumentation, spectrophotometer, and PH meter. Salary range is $26,600 to $32,000 per year for a 35 hour work week. Qualified applicants respond with resume only to: **J.O. 0559973, Job Service Technical Unit, Connecticut Department of Labor, 200 Folly Brook Blvd, Wethersfield, Connecticut 06109.** (NW620)A

MEDICAL RESEARCH COUNCIL

Clinical Oncology and Radiotherapeutics Unit,

AREER NON-CLINICAL SCIENTIST BIOCHEMIST/ CELL BIOLOGIST

pplications are invited for a
reer non-clinical scientist post
ith the Medical Research Coun-
l. The post is tenable at this Unit
the first instance. The successful
plicant will be expected to work
factors which control tumour
ll sensitivity to chemotherapeu-
agents and radiation, with par-
ular interest in the biochemis-
y, molecular and cellular biology
the processes of DNA damage
d repair. The post will be an in-
gral part of the Unit's research
ogramme which comprises cel-
ar and pharmacological studies
drug resistance and metabol-
m, tumour marker expression,
ermochemotherapy, oncogene expression and novel applications of flow cytometry. The successful applicant will have a background in cellular biology and/or nucleic acid research and will have demonstrated the ability to lead independent research projects and initiate scientific collaborations.

Salary will be at the appropriate point on the scale for non-clinical scientific staff. Applications (6 copies), including a full curriculum vitae, with list of publications and the names of 3 referees should be lodged by 26 March 1987 with the Director, Professor N M Bleehen, MRC Clinical Oncology and Radiotherapeutics Unit, MRC Centre, Hills Road, Cambridge CB2 2QH, from whom further details may also be obtained.

Applicants should include a statement of about 1,000 words in length explaining the research programme that they would wish to carry out if appointed.

The MRC is an Equal Opportunities Employer. (2354)A

The Ohio State University

THE DEPARTMENT OF MICROBIOLOGY

invites applications for a tenure track Assistant Professorship in Virology. Candidates with interests in an aspect of viral disease or disease prevention are particularly encouraged to apply, however candidates with expertise in any viral system will be considered. The successful candidate must have demonstrated their research potential by recent publications and will be expected to develop and maintain an independent research program, teach an undergraduate course in general virology, participate in the general teaching responsibilities of the Department and train graduate students. Candidates should have a Ph.D. degree and at least 2 years of postdoctoral experience.

Candidates should submit a curriculum vitae, a summary of research plans and the names of three references to: Dr Bruce S. Zwilling, Chairman, Virology Search Committee, Department of Microbiology, College of Biological Sciences, The Ohio State University, 484 West 12th Avenue, Columbus, Ohio 43210. The position is available starting Autumn 1987, applications received before April 15, 1987, will be considered in the first instance however applications will be accepted until the position is filled.

The Ohio State University is an Affirmative Action/ Equal Opportunity Employer (NW621)A

AUSTRALIA

CSIRO

DIVISION OF TEXTILE INDUSTRY

BELMONT VIC

GENERAL: The Division of Textile Industry, with a staff of 200 including 60 professional scientists, forms part of the Institute of Industrial Technology. The role of the Division is to increase world demand for Australian wool by carrying out multidisciplinary research to improve wool processing and the attractiveness of the wool end-product. The Division has an excellent record of translating research results through to commercially successful products and processes. As part of a network of research establishments and industry, there are excellent opportunities for collaboration with local and overseas collegues conducting research in related fields. The Division possesses well-equipped laboratories, engineering workshops, electronics and computing support groups and a full-scale textile mill. The research planned for the future requires creative R & D professionals who are prepared to work on intellectually demanding projects in support of global strategic marketing strategies for Australian wool. The following positions are immediately available.

RESEARCH SCIENTIST (PHYSICAL CHEMISTRY)

A$28,107 — A$41,339

Reference No. C8576

The successful applicant will undertake an investigation of the causes of dimensional changes that occur in wool fabrics as a result of moisture sorption, and the effects of these changes in practice.

RESEARCH SCIENTIST (PHYSICAL CHEMISTRY)

A$28,107 — A$41,339

Reference No. C8581

The successful applicant will undertake an investigation of the role of the various morphological components of wool fibres in the chemical processing of wool textiles. Initially, studies will centre upon the diffusion of dyestuffs into the cortical cells of the wool fibre.

QUALIFICATIONS: Applicants must have a PhD or postgraduate research experience of equivalent standard. A good background in physical chemistry is essential.

TENURE: Indefinite with Australian Government superannuation benefits available.

SALARY LEVEL: Research Scientist/Senior Research Scientist. Appointment at a higher level may be considered for an outstanding applicant.

APPLICATIONS: Further technical details may be obtained from Dr R. Garnsworthy (052-472672). Applications stating relevant personal details, details of qualifications and experience, the names of at least two professional referees and quoting the appropriate reference number should reach:

The Chief
CSIRO Division of Textile Industry
PO Box 21
BELMONT VIC 3216
AUSTRALIA.

by April 10, 1987.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W3904)A

POSTDOCTORAL RESEARCH ASSOCIATE

A position is available for individual interested in cellular neurophysiology. Actions of inhibitory and excitatory amino acid neuro-transmitters will be studied using *in vitro* brain slice preparations and dissociated cell cultures. Prior experience with intracellular or whole cell patch recording techniques required. Funding is available for a minimum of two years.

Interested candidates should send curriculum vitae and names of two references to: **Dr John J. Hablitz, Department of Neurology, Baylor College of Medicine, Houston, Texas 77030.** (NW626)A

Synchrotron Radiation Research in Biophysics at Chess

A substantial expansion of the synchrotron radiation facilities at CHESS, the Cornell High Energy Synchrotron Source, will shortly occur, through the construction of new undulator, wiggler and bending magnet beam lines. Three senior positions funded by MacCHESS, the NIH Biomedical Technology Program at CHESS, are open in the general area of diffraction from biological macromolecules. We are particularly interested in the development of Laue diffraction techniques and time-resolved crystallography (see *Science 223,* 1423-1425 (1984); *Nucl. Instr. Meth. 222,* 245-251 (1984); *Nucl. Instr. Meth. 246,* 627-635 (1986).

Senior Research Associate: Overall responsibility for the design, construction and testing of synchrotron radiation instrumentation — x-ray optics, cameras, sample handling devices, detectors — together with associated software for data acquisition and reduction. Applicants must hold a PhD in a physical or chemical science, and have at least four years experience in the design and construction of advanced diffraction instrumentation, preferably at a synchrotron x-ray source, with supervisory experience. Further experience in biophysical chemistry or biochemistry is highly desirable.

Research Associate: Together with the Senior Research Associate, responsible for aspects of the development and use of novel diffraction instrumentation, particularly in the area of Laue diffraction from single crystals of macromolecules. Applicants must hold a PhD in a physical, chemical or biological science, and have at least two years experience in diffraction instrumentation of software, macro-molecular crystallography, or related fields.

Postdoctoral Associate: Together with the senior staff, responsible for the development of enzyme or other macromolecular systems suitable for time-resolved crystallography, using Laue diffraction techniques. Applicants must hold a PhD in a chemical or biological science, with a background in enzymology or macromolecular crystallography.

All three positions will assist outside users of MacCHESS in the collection of accurate diffraction data. Salary depends on experience; starting salaries will be at least $30,000 for the Senior Research Associate, $25,000 for the Research Associate, and $20,000 for the Postdoctoral Associate.

Applicants should send a c.v., list of publications, and the names of three referees before May 8 to **Professor Keith Moffat, Director of MacCHESS, 252 Clark Hall, Cornell University, Ithaca, New York 14853, U.S.A.** Further information may be obtained from him, or from Professor Boris Batterman, Director of CHESS, Wilson Laboratory, Cornell University.

Cornell is an Affirmative Action/Equal Opportunity Employer. (NW613)A

TRANSPLANTATION IMMUNOLOGIST

The Cleveland Clinic Foundation invite
applications for a full-time position invol
ing a primary appointment in the Depar
ment of Immunology & Cancer and a joir
clinical appointment. Interested appl
cants must have an MD and/or PhD degre
with considerable postdoctoral exper
ence in basic transplantation immun
logy. Candidates must be establishe
investigators with evidence of hig
quality independent research, and a co
sistent history of external funding su
port. Essential qualities of the successf
applicant are the ability to develop
research program bridging basic labor
tory investigation with relevant problem
in transplantation science, and a willin
ness to interact with basic scientists
well as transplant clinicians. Pleas
submit a letter of application, curriculu
vitae, and research interests to:

Andrew C. Novick, M.D.
Chairman, Department of Urology

THE CLEVELAND CLINIC FOUNDATIO
9500 Euclid Avenue Cleveland, Ohio 44106

The Cleveland Clinic Foundation is an Equal Opportunity/Affirmative Action Employer.

(NW608)A

THE UNIVERSITY OF ESSEN MEDICAL SCHOOL

is seeking a

FULL PROFESSOR (C4)

OF CELL AND MOLECULAR BIOLOGY CANCER RESEARCH

for the Institute of Cell Biology (Cancer Research) at the West German Cancer Center, Essen

The Institute of Cell Biology (Cancer Research) is currently undergoing an extension of staff and laboratory facilities. Research is focused on the molecular and cellular basis of carcinogenesis, on the control of cell proliferation and differentiation, and on experimental studies relevant to cancer prevention, diagnosis and therapy, with emphasis on developmental biology.

Applicants are expected to participate in the rotating chairmanship of the institute and to cooperate closely with the other research groups of the institute and with the institutions and clinics of the Medical School; to participate in the Sonderforschungsbereich 102 ("Experimental and Clinical Leukemia and Tumor Research") of the Deutsche Forschungsgemeinschaft at University of Essen Medical School; and to contribute to the institute's teaching obligations. PhD and/or MD degree is required as well as high standard scientific qualifications in the institute's area of research.

Applications, along with full personal and professional details, should be sent to: **Dekan des Fachbereiches 14 (Medizin) der Universität Essen (GH), Universitätsklinikum Essen, Hufelandstraße 55, D-4300 Essen 1, Federal Republic of Germany.** Closing date for receipt of applications 20 April, 1987. (W3900)A

UNITED MEDICAL AND DENTAL SCHOOLS

SENIOR LABORATORY SCIENTIFIC OFFICER

Senior Laboratory Scientific Officer required in the Department of Anaesthetics at our Guy's Campus, London Bridge.

He, or she, will be expected to play a part in the Department's clinical and basic science research programme which is directed towards all aspects of improving the safety and care of patients undergoing surgery.

The successful candidate will have the opportunity to learn new skills and use initiative, working in a team in excellent surroundings. The department is well supported with word processing and computer facilities.

For further information, please contact Professor A. P. Adams, or Dr R. M. Jones on 01-407-7600, extension 2098.

Salary on Whitley scale £8,346—£11,026 plus London Weighting Allowance of £1,201 per annum.

Applications with full curriculum vitae and the names and addresses of two referees, should be sent to the Staffing Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH, quoting reference G/ANAES/140. (2304)A

MEDICAL RESEARCH COUNCIL

NATIONAL INSTITUTE F MEDICIAL RESEARC

DIVISION OF PARASITOLOG

A JUNIOR POSTDOCTORAL POSITION

funded by the Edna McCo
Clark Foundation is availabl
work on *Schistosoma Haem*
bium Antigens for a period of u
two years. The project to
undertaken involves the id
fication and characterisatio
cDNA clones encoding anti
recognised during rodent
human infections as well as a
sis of their role in prote
immunity. Previous experien
working with schistosoma m
ular biology or immunochem
will be an advantage. Salary w
in the range £9,495—£10,375
award pending) plus £1
London Allowance per an
with optional entry to the
Pension Scheme.

Please send their applicatio
Mr C. R. Russell, Head of
ministration, NIMR, The Ri
way, Mill Hill, London NW7
enclosing full c.v. and the nam
two referees by Friday 3rd A
1987 quoting reference PA
An equal opportunities empl
(2341

INSTITUTE OF CANCER RESEARCH

A vacancy exists for a

TECHNICIAN/RESEARCH OFFICER

o work in the Radiotherapy Research Laboratories at Sutton, Surrey. The successful applicant will join a team who are investigating the sensitivity of human tumour cells to irradiation, seeking to relate cell survival to underlying processes of cellular damage. Experience in tissue culture or the measurement of DNA damage would be an advantage.

Research Officer salary: £7,872–£10,101 inclusive of London Weighting.

Technician salary: £7,045—9,663 inclusive of London Weighting.

Applicants are advised that in the majority of the Institute's premises smoking is prohibited.

Applications, in duplicate, with the names of two referees, to the Personnel Officer, The Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting reference 3/87/T/NA/64.

(2334)A

UNIVERSITY OF LONDON CHAIR OF PSYCHOLOGY AT ROYAL HOLLOWAY AND BEDFORD NEW COLLEGE

The Senate invite applications for the above Chair. Applications (10 copies) should be submitted to the Teachers' Section (N), University of London, Senate House, Malet Street, London WC1E 7HU, from whom further particulars should first be obtained.

The closing date for receipt of applications is 8 April 1987.

(2314)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL FELLOW

Applications are invited from Biochemists (or related disciplines) for the study, with Professor A. B. Cooke, of the mechanisms of luteinzing hormone induced desensitization of adenylate cyclase and receptor recycling and down regulation.

MRC funded post available for three years. Salary on Research 1A scale.

Applications in the form of a full curriculum vitae, including the names and addresses of two academic referees, should be sent to the School Office, RFHSM, Rowland Hill Street, London NW3 2PF (01-794-0500, extn 4262). Closing date 27 March 1987. Please quote reference BAC/PFB/2. (2333)A

ASTON UNIVERSITY

DEPARTMENT OF VISION SCIENCES

ROAD SIGNAL PERCEPTION GROUP

CONTRACT RESEARCH FELLOW

A vacancy has arisen for a colleague with leadership ability and drive to join a team carrying out research into the conspicuity and information efficiency of road traffic signs and signals. Previous studies have successfully been undertaken by the Road Signal Perception Group over the past five years.

The Group is part of the Department of Vision Sciences whose research was judged, by the University Grants Committee, to be "outstanding" on an international basis.

The post will be for a period of one year initially. Commencing salary will be within the range £8,020 to £11,790 per annum (presently under review), depending upon age, qualifications and experience.

For more information and an informal visit to the Department of Vision Sciences contact Peter Linfield, telephone 021 359 3611, extension 5162.

Application forms and further particulars may be obtained from the Personnel Officer (Academic Staff, (quoting Ref: 875/6), Aston University, Aston Triangle, Birmingham B4 7ET. Tel: 021 359 0870 (24-hour answerphone). Closing date for the receipt of applications is 10th April 1987.

(2306)A

DEPARTMENT OF APPLIED MATHEMATICS AND THEORETICAL PHYSICS

TEMPORARY APPOINTMENT

Applications are invited for a temporary academic appointment in the Department with both teaching and research duties for the period 1 October 1987 to 30 September 1990, with the possibility of reappointment for a further period to 30 September 1992, to provide a replacement for Dr N. O. Weiss who will be on leave during that period. Applicants will be considered for most branches of applied mathematics and theoretical physics. The person appointed will be expected to take up appointment on 1 October 1987, and to undertake teaching duties similar to those of a University Lecturer in the Department. The salary will be on the age-related scale for University officers.

Further information may be obtained from Professor J. C. Taylor, Deputy Head of the Department of Applied Mathematics and Theoretical Physics, Silver Street, Cambridge, CB3 9EW.

(2311)A

Group Microbiologist

Top Salary + Car

Burton upon Trent, Staffordshire

Bass, Europe's largest brewing group, are looking for a microbiological specialist, directly responsible to the Quality Control Director. He or she will provide an advisory and trouble-shooting service to all Bass Breweries and manage the activities of the Group QC Laboratory.

You will supervise the Bass Yeast Quality Assurance Policy, advise Breweries in respect of yeast propagation systems and yeast husbandry, and provide an advisory and auditing service to Brewery microbilogical schedules. You will also assess new/improved microbiological techniques to meet specific QC needs.

You must have a Ph.D. in Microbiology and at least five years post graduate experience in the brewing/fermentation industries.

Salary will be commensurate with age and experience. This position offers good career progression opportunities with a first class Company.

Write now for application form to:
Personnel Services Manager,
Bass Public Limited Company, 137 High Street,
Burton-on-Trent, Staffordshire, DE14 1JZ, or
telephone 0283 45301, x 2511. (2355)A

Bass
Public Limited Company

OXFORD POLYTECHNIC

Faculty of Technology
Department of Biology

Postdoctoral Research Fellow — Researcher B scale £8,595 — £13,656.

Research Assistant — Researcher A scale £6,060 — £8,196.

Applications are invited for the above posts, which will be available from 1 April 1987, to join a team working on the development of immunoassay methods for analytes of clinical importance in collaboration with Boots-Celltech Diagnostics Limited. The posts are funded by the NAB Research Initiative and are for one year, renewable for two further years.

Further particulars and application forms can be obtained from the **Staffing Office, Oxford Polytechnic, Gypsy Lane, Headington, Oxford OX3 0BP** Telephone Oxford 819052/819055. *Closing date: 27 March 1987.*

(2322)A

Postdoctoral Positions

Molecular Biology

The Department of Molecular Biology at GENENTECH, INC. has institutionally funded positions for postdoctoral fellows to work in the area of molecular and cellular biology of the blood vessel wall. The research will focus on interactions of growth factor with endothelial and smooth muscle cells on the regulation of firbrinalysis, and the neovascularization process. Duration of appointments will be 2-3 years.

Candidates should submit curriculum vitae with three references to: **Dr R. Derynck, Senior Scientist, GENENTECH. INC, 460 Point San Bruno Blvd., South San Francisco, CA 94080.**

An Equal Opportunity Employer. (NW624)A

SENIOR-PROFESSOR POSITION

at the Centre de recherche en reproduction animale and the Département d'anatomie et physiologie of the *FACULTÉ DE MÉDECINE VÉTÉRINAIRE* of the Université de Montréal is seeking a full professor.

Duties: Teaching undergraduate courses in reproduction; establishing research projects at the Centre de recherche en reproduction animale; supervision of graduate students.

Requirements: D.V.M. and PhD or equivalent; ability to work in a French environment; experience in recruiting and supervising graduate students at the Masters and PhD level. Candidates must show evidence of strong research achievements in animal reproduction, especially in the area of biotechnology.

Salary: Commensurate with qualifications and experience.

Starting date: To be determined.

Candidates should send their curriculum vitae together with a letter demonstrating their interest for the position, copies of appropriate academic diplomas and the names of three referees, before May 1st, 1987 to: **Monsieur Raymond S. Roy, doyen, Faculté de médecine vétérinaire, Université de Montréal, C.P. 5000, Saint-Hyacinthe (Québec), Canada, J2S 7C6. Tél.: (514) 773-8521,**

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. (NW615)A

FRIEDRICH MIESCHER-INSTITUT

Applications are invited for the position of a

GROUP LEADER IN PLANT BIOLOGY

Candidates should have a strong research background in Plant Sciences at the cell and/or molecular level, as well as a demonstrated ability to develop an independent research programme and to lead a group.

The candidate will be expected to carry out research in an area complementing the work of five other group leaders: Witek Filipowicz (gene expression and RNA processing), Barbara Hohn (genetic flux in plants), Thomas Hohn (molecular plant virology), Patrick King (a genetic approach to plant hormones), and Frederick Meins (plant development).

The groups each consist of postdoctoral fellows, graduate students and technical staff, and occupy independent laboratories.

We would prefer to make the appointment at the level of a senior group leader, but an appointment at a more junior level is not excluded.

A curriculum vitae and a list of publications should be submitted before March 31st, 1987 to: **Search Committee (Plant Group Leader), Friedrich Miescher-Institut, PO Box 2543, 4002 Basel, Switzerland.**

(W3890)A

L'UNIVERSITE DE LAUSANNE

ouvre une inscription pour la pourvue d'un poste d'

agrégé de Faculté à l'Institut d'astronomie

Entrée en fonction:
1er septembre 1987

Le titulaire participera à l'enseignement et aux recherches photométriques (notamment collaboration avec le Centre de Données Stellaires de Strasbourg) de l'Institut.

Les candidatures (avec curriculum-vitae, liste des publications, tirés-à-part des cinq publications les plus significatives et références) seront adressées **jusqu'au 31 mars 1987** au Doyen de la Faculté des sciences, Collège propédeutique, CH-1015 Lausanne.

Renseignements auprès du prof. B. Hauck, Directeur de l' Institut d'Astronomie, 1290 Chavannes-des-Bois.

(W3888)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF RADIATION ONCOLOGY

RESEARCH ASSISTANT/ SENIOR RESEARCH ASSISTANT

Applications are invited for the above post to work on resistance to alkylating agents in human cancer cells. A background in biochemistry or cell biology would be an advantage and experience in tissue culture essential. The project is funded by the Cancer Research Campaign for a three year period. The laboratories are situated at Clatterbridge Hospital, Wirral and have recently been refurbished and equipped to a high standard. Informal enquiries may be made to Dr J. A. Green, Tel. 051-334 4000 Ext. 4177.

Initial salary will be on the scale £7,055—£9,495 per annum (under review). Applications, together with the names of three referees, should be received not later than 30th March 1987, by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/376/N.

(2318)A

CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE

Whole Plant Physiology

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a one year miminum position to study environmental effects on the physiology of water hyacinth with the aim of building a whole plant function model. Experience would be useful in areas of mineral nutrition /hydroponics and modelling. Applicants should submit a c.v. and 2 letters of recommendation to Jacques Roy Center Emberger B.P. 5051/34033, Montpellier Cedex France. (W3902)A

POSTDOCTORAL POSITION

Available Spring 1987, University of California, San Francisco. To study the structure and function of polypeptide hormone receptors. Requires background and interest in protein purification, immunology, and peptide chemistry. Experience with HPLC and hybridoma techniques preferred. Send curriculum vitae and names of three references to: **Dr Carl Grunfeld, Metabolism Section (111F), VA Medical Center 4150 Clement St. San Francisco, CA 94121.**

UCSF is an equal opportunity/ affirmative action employer; women and minorities are encouraged to apply. (NW629)A

UNIVERSITY OF EDINBURGH

EDINBURGH SCHOOL OF AGRICULTURE

RESEARCH ASSISTANT O ANIMAL BEHAVIOUR

Applications are invited for a po in the Animal Division to carry o research on Housing Systems f laying hens. Initial studies indica that it is possible to modify co ventional cages to improve t welfare of hens while retainir economic competitiveness. Fun are available from MAFF for tw years for more detailed work the behavioural response of he to such modifications. Candidat should hold a relevant first degr and have adequate postgradua experience. Starting salary will £6,711 per annum.

Further particulars and applic tion forms may be obtained fro the Secretary, the Edinburg School of Agriculture, West Mai Road, Edinburgh EH9 3JG. R ference A8/MAFF should quoted in all communication Closing date for applications: 30 March 1987. It is hoped that t appointment will start on 1 Ma 1987. (2327)A

UNIVERSITY OF LONDON

CHAIR OF GENETICS AT KING'S COLLEGE LONDON (KQC)

The Senate invite applications f the above newly-establishe Chair, tenable in the Faculty Life Sciences at King's Colleg London (KQC). Candidat should have research interests in field of Molecular Genetics.

Applications (10 copies) shoul be submitted to the Teacher Section (N), University of Lo don, Senate House, Malet Stree London WC1E 7HU, from who further particulars should first b obtained.

The closing date for receipt applications is 15 April 1987.

(2326)A

MOLECULAR BIOLOGY POSTDOCTORAL IN PROTEIN BIOSYNTHESIS

NEEDED: PhD researcher who can combine kinetic studies on translation with recombinant DNA route to RNA substrates. Read: JMB *192*, 235 (1986) and JBC *261*, 4868 (1986). Write: **Mike Yarus, Dept MCD Biology, Box 347, University of Colorado, Boulder, CO 80309.**

University of Colorado at Boulder has a strong institutional commitment to the principle of diversity. In that spirit, we are particularly interested receiving applications from a broad spectrum of people, including women, members of ethnic minorities and disabled individuals. (NW633)A

UNIVERSITY OF DUBLIN TRINITY COLLEGE

LECTURER IN APPLIED MATHEMATICS

Applications are invited for a vacant lectureship in the Department of Applied Mathematics in Trinity College, Dublin. The Departments of Pure and Applied Mathematics (which together form the School of Mathematics) are responsible for the moderatorship (honor) course in Mathematics and for the Mathematics component in various joint moderatorship courses. The two departments also have substantial teaching responsibilities to students in Science and Engineering to which the successful candidate will be expected to contribute.

The research interests of the department include theoretical physics (quantum field theory and particle physics), general relativity, fluid dynamics (boundary layers), non-linear and inverse problems, theoretical computation. Applicants willing to develop inter-disciplinary research will be particularly welcomed; there are widespread opportunities for such research throughout the Science and Engineering Faculties. Appropriate research areas include non-linear and complex systems, computation, network theory, and image processing; applications from candidates in other areas of Applied Mathematics and Theoretical Physics will also be welcomed.

Salary Scale: IR£10,065—IR £19,922 per annum. Appointment will be made within the salary range IR£10,065—IR £14,220 p.a. at a point commensurate with qualifications and experience to date.

Application forms and further particulars relating to this post may be obtained from: **Establishment Officer, Staff Office, Trinity College, Dublin 2.**

The closing date will be Friday, 27th March, 1987. (2305)A

POSTDOCTORAL POSITION

to study a gene encoding a kidney tumor specific antigen. The project is funded by the German Research Foundation (DFG) for the period July 87 till December 89. Salary DM 50,000—60,000 (BAT IIa), depending upon age and experience.

This is a joint project between the Department of Urology, Medical School, Mainz and the Institute of Toxicology, Mainz. Applications including a curriculum vitae and the names of two referees should be sent to **Dr J. Doehmer, Institute of Toxicology, University of Mainz, Obere Zahlbacher Str. 67, 6500 Mainz.** (W3896)A

The GBF — a government supported National Research Institute of Biotechnology, located at Braunschweig, Lower Saxony — is searching for a scientist to head a new group in the area of

Cell biology or Immune biology

The candidate should have an excellent research record and be in a position to lead a group and create his own research program. The salary will be in analogy to a C4-Professorship.

The attractiveness of this position lies in the manifold possibilities to collaborate with the other GBF-research groups working on

Eukaryotic and procaryotic genetics
Protein design
Large scale tissue culture and
Molecular biology of biologically active peptides

Possibilities to interact with the academic teaching and research program at the Technical University are also provided.

Please send c.v. and two recommendations in all confidence to **Prof. Dr J. Klein, Scientific Director of Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, D-3300 Braunschweig, FRG, until April 15, 1987.**

If required, please call Professor Dr J. Collins, Head of Cell Biology and Genetics Division of GBF (531-6181-200) for more information. (W3910)A

POSTDOCTORAL ASSOCIATE

A postdoctoral appointment of up to three years' duration is available for research on the biosynthesis of steroids and steroid hormones. Please send curriculum vitae and the names of three references to: **Dr. Eliahu Caspi, c/o Personnel Office, Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545, USA.**

An Equal Opportunity/ Affirmative Action Employer H/F/H.

(NW622)

POSTDOCTORAL POSITION

in Molecular Biology of Epithelial Differentiation

Available summer 1987 to study the molecular biology of epithelial cell surface molecules and other differentiation antigens (J. Cell Biol. 103: 41 and 49, 1986). Experience in recombinant DNA essential. Send resume and 3 letters of reference to: **Drs Tung-Tien Sun and Warren Jelinek, Departments of Dermatology and Biochemistry, New York University Medical School, 550 1st. Avenue, N.Y., N.Y. 10016.** (NW612)A

AUSTRALIA
CSIRO
RESEARCH FELLOW
A$28,107 – A$41,339
DIVISION OF FOOD RESEARCH
DAIRY RESEARCH LABORATORY
HIGHETT VIC

FIELD: Concentrated and Dried dairy Products.

GENERAL: The Dairy Research Laboratory located at Highett forms part of the Division of Food Research with its headquarters at Ryde, NSW.

The Laboratory conducts basic and strategic research programs directed towards the improvement of existing dairy products and processes and the development of new product areas for the dairy industry.

DUTIES: As a member of a group to undertake research in the physical, chemical, functional and organoleptic properties of concentrated and dried products resulting from adjustment of the mineral and/or protein levels of these products. These adjustments may be induced by the manipulation of processing variables, direct introduction of protein or mineral isolates or through the application of membrane technology.

It is anticipated that the appointee will be in a position to develop products with specific characteristics and to liaise with industry in this field.

QUALIFICATIONS: A PhD degree in Science, Applied Science or equivalent qualifications. Previous experience in the food industry is desirable.

TENURE: A term of 3 years, with the possibility of a further term. Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No: A4025, should be directed to:

The Chief
CSIRO Division of Food Research
PO Box 52
North Ryde 2113
AUSTRALIA

By April 10, 1987.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W3903)A

UNIVERSITY DEPARTMENT AND MRC UNIT OF CLINICAL PHARMACOLOGY OXFORD

POSTDOCTORAL NEUROSCIENTIST

is required for studies on the central and peripheral neuropharmacological mechanisms of emesis produced by cancer chemotherapeutic agents and irradiation. The methodology which will be applied will include [C^{14}]-2-Deoxyglucose autoradiographic visualisation of regional neuronal activity in the brain stem during emesis in experimental animals. A general interest in autoradiographic techniques with densitometric analysis and their application to functional neuropharmacological problems is desirable. There is technical assistance, the techniques are established and full instrumentation available.

Applicants should have postdoctoral experience in an appropriate field. The post is for three years on salary scale of University Research Scientist Grade 2.

Further information may be obtained from: **Professor D. G. Grahame-Smith, University Department of Clinical Pharmacology, Radcliffe Infirmary, Woodstock Road, Oxford, OX2 6HE,** to whom applications should be sent by the closing date of 6th April, 1987. (2319)A

POSTDOCTORAL OR RESEARCH ASSOCIATE POSITION

Available immediately for 2 to 3 years to participate in studies involving molecular cloning and expression of falciparum malaria genes. Should have some experience with biochemical techniques. Salary negotiable, depending on experience. **Send curriculum vitae and three letters of reference to: Dr. A.C. Vezza, Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294.** An Affirmative Action/ Equal Opportunity Employer.

(NW602)A

ANATOMY

UNIVERSITY OF SASKATCHEWAN

Department of Anatomy

The Department of Anatomy, University of Saskatchewan, College of Medicine, invites applications for tenure-track position at the Assistant Professor level. Applicants should have experience in teaching gross anatomy to medical and dental students as well as neuroanatomy and embryology. Applicants should have proven research background and a strong research program. Application, including curriculum vitae, names of three referees and an outline of proposed research should be submitted to Dr S. Fedoroff, Head, Department of Anatomy, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, S7N OW0.

(NW609)A

MEDICAL RESEARCH COUNCIL

POSTDOCTORAL BIOCHEMIST/MOLECULAR BIOLOGIST

A short-term (3 years) position is available in the Blood Pressure Unit to work on the control of synthesis and release of the enzyme renin by hormonal and other factors. Initial salary on point 4 of the scale for non-clinical scientific staff. Applications to be sent to Dr Brenda Leckie, MRC Blood Pressure Unit, Western Infirmary, Glasgow, G11 6NT by 31st March, 1987. (2332)A

UNIVERSITY OF GLASGOW

BURTON CHAIR OF NEUROLOGY

Applications are invited for the above Chair which will fall vacant on 1st October, 1987 on the retiral of Professor J. A. Simpson. The Chair will be endowed by The Burton Fund in accordance with the Resolution of the University Court.

Medically qualified candidates will receive an appropriate honorary post with the Greater Glasgow Health Board.

The post is located in the Department of Neurology at the Institute of Neurological Sciences at the Southern General Hospital, Glasgow.

Further particulars may be obtained from the Academic Personnel Office, University of Glasgow, Glasgow, G12 8QQ, where applications (3 copies; 1 copy in the case of overseas applicants), giving the names and addresses of three referees, should be lodged on or before 17th April, 1987.

In reply please quote Ref. No. 5898M. (2328)A

UNIVERSITY OF SURREY

Department of Microbiology

POSTDOCTORAL RESEARCH FELLOW FOR THE DOWNSTREAM PROCESSING OF MAMMALIAN CELL CULTURE FLUIDS

A position is available for a postdoctoral Biochemist/Biotechnologist who has had some experience in the separation of proteins from culture supernatants. The applicant should be capable of designing a screening system to determine the set of procedures which will lead to the most efficient concentration and purification practices. Also he/she should be able to apply such procedures to a number of bioprocesses running in parallel. This work requires a person who will fit into a team of some eight people who are investigating alternative bioprocess systems. A member of this team will be dedicated for the technical support of the successful applicant.

Salary will be on the Research and Analogous Staff scale between £8,020 and £13,800 per annum depending on qualifications, age and experience. The post is for two years in the first instance with prospects for renewal on a year to year basis. Superannuation under USS conditions.

Applications in the form of a CV (3 copies) and the names and addresses of 2 referees should be sent to (JLG) Personnel Office, University of Surrey, Guildford, GU2 5XH, quoting Ref No: 603/N by 3 April 1987. (2339)A

THAMES POLYTECHNIC

Incorporating Avery Hill College

SCHOOL OF BIOLOGICAL SCIENCES & ENVIRONMENTAL HEALTH

READER

Applications are invited from appropriately qualified biologists with experience in areas of work closely related to the School's present biological interests. The School has considerable research activity and the main areas are the biochemistry of bacterial cell walls, lignin degradation, protein biochemistry, affinity chromatography, renal physiology and yeast genetics.

Salary scale: £15,894 — £17,568 (bar) — £19,698 inclusive.

Further particulars and application form from the Staffing Officer, Thames Polytechnic, Wellington Street, London SE18 6PF to be returned by 24 March 1987.

Thames Polytechnic is an equal opportunities employer.

(2346)A

UNIVERSITY OF LIVERPOOL

Department of Radiation Oncology

Clatterbridge Hospital,
Bebington, Wirral

RESEARCH ASSISTANT

Applications are invited for th post of Research Assistant to wo in the Department of Radiati Oncology on a Cancer Researc campaign funded project radiosensitizer targetting. Th work will involve tissue cultur preparation of antibod radiosensitizer conjugates, dete mination of dose-response activi by clonogenic assay and biochem cal investigations of cell uptal and metabolism of conjugate Applicants should be good Ho ours graduates in Biochemist with relevant laboratory exper ence.

The post is tenable for one ye in the first instance at an initi salary within the range £7,055 – £9,495 per annum (under review

Applications together with th names of three referees should b received not later than 30 March, 1987 by The Registra The University, PO Box 14 Liverpool L69 3BX, from who further particluars may b obtained.

Quote ref RV/379/N.

(2358)A

NATURE CONSERVANCY COUNCIL

Ref: HE2-36-77

SCIENTIFIC OFFICER

FRESHWATER BOTANIST

Temporary Post

The NCC requires a botanist t join a team surveying freshwate lochs in Northern Scotland. Th appointment will be for one yea initially with a possibility o extension for a further six months The post is based in Edinburg and will be pensionable.

Qualifications: You must have good degree in botany or othe relevant biological subject. Exper ience of freshwater survey work good working knowledge of wate plants and a current driving licenc are all essential to this post.

Salary: £6,711 to £9,22 depending upon qualifications an experience.

Field Assistants: In addition t the above post, a team of 4 scien tific officers is required to assis with botanical field surveys o freshwater lochs. These will b employed for up to 3 month between June and October 1987 Qualifications as for Scientfi Officer.

Application forms and furthe particulars available from Per sonnel I, The Nature Conservanc Council, Northminster House Peterborough PE1 1UA. Th closing date for completed appli cations is 31 March 1987. Th NCC is an equal opportunit employer. (2040)A

UNIVERSITY OF ZIMBABWE

LECTURESHIPS/SENIOR LECTURESHIPS/ ASSOCIATE PROFESSORSHIPS

Department of Biochemistry

pplicants should have a post-raduate degree, preferably a PhD n a speciality in Nutrition and with strong background in Biochemstry. University teaching experence would be an advantage. The ppointee will be expected to: (i) each short courses in nutritional iochemistry to science, medical nd veterinary students; (ii) develop and organise full nutrition ourses at university level; (iii) nitiate a programme of research n his/her area of interest; (iv) upervise postgraduate students.

Department of Biological Sciences
(2 Posts)

Post A: A Lecturer/Senior Lecturer is sought to provide basic ndergraduate courses in General Zoology. A PhD or equivalent esearch experience would be the ormal minimum qualification.

Post B: A Lecturer/Senior Lecturer is sought to provide basic ndergraduate courses in General Botany. A PhD or equivalent esearch experience would be the ormal minimum qualification.

Salary Scales — Lecturer Z$18,216 - 26,832, Senior Lecturer Z$27,732 - 30,360, Associate Professor Z$31,284 - 2,640. Appointment on the bove scales according to qualifications and experience.

Conditions of Service: Both ermanent and short-term contracts are offered. Persons who are ot Zimbabwean citizens may be ppointed only on a short-term ontract basis for an initial period of two years. Short-term contracts may be extended.

Six Copies of applications, giving full personal particulars, which should include full name, place and date of birth, qualifications, employment and experience, present salary, date of availability, telephone number and names and addresses of three referees, should be addressed to the Director, Appointments and Personnel, University of Zimbabwe, PO Box MP 167, Mount Pleasant, Harare, Zimbabwe (telex: 4-152 ZW). Candidates in UK should also send an additional copy of their applications to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom further particulars may be obtained. Closing date for receipt of applications is 10 Aril 1987.

(W3908)A

SUNY-GENESEO
Department of Biology
ASSISTANT PROFESSOR

for electron microscopy (EM) and cell biology courses. Must maintain Departmental EM facility and develop research program. Requires PhD. Position starts September 1987. Apply by April 20 to: **Dr R. Simon, Department of Biology, State University of New York (SUNY)-Geneseo, Geneseo, NY 14454, USA.** *Affirmative Action/Equal Opportunity Employer.*

(NW631)A

UNIVERSITY OF WARWICK
RESEARCH TECHNICIAN

The Department of Biological Sciences has a vacancy for a Technician in the Microbiology Research Group. The work is involved with the physiological and biochemical aspects of n-alkene utilization by microorganisms. The post is available for a contract period of two years.

Applications are invited from graduates and others with qualifications and experience in biochemistry and/or microbilogy.

Salary on Technician grade 3 scale: £5,968 — £7.040 pa.

Application forms from the Personnel Office, University of Warwick, Coventry CV4 7AL (0203 523627) quoting Ref No 33/T/86/0. Closing date 26th March 1987.

An equal opportunity employer. (2348)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
(University of London)

DEPARTMENT OF IMMUNOLOGY

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a postdoctoral research fellow to work in the molecular immunology group of Dr R. Lechler. The proposed research project will involve exploration of the role of MHC Class II molecules in the generation of the T lymphocyte repertoire. The research will require use of molecular (gene cloning) and cellular techniques. Applicants with experience and recombinant DNA, and/or cellular immunology are particularly encouraged to apply.

Salary scale will be £9,000—£10,000 plus £1,393 London Allowance a year.

Application forms may be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0HS quoting reference AIA8. Closing date: 26th March 1987. (2315)A

MEDICAL RESEARCH COUNCIL

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

Scientific appointments in the Divisions of Virology and Immunology

Applications are invited for two posts for work in the field of human retroviruses at the National Institute for Medical Research: a Career Non-Clinical Scientific appointment and a Short-Term Non-Clinical Scientific appointment.

The career appointment is for work on the genetic diversity of human immunodeficiency viruses in the Division of Virology (Ref: V/871)

The short-term appointment is for work on the recognition of HIV glycoproteins by immune T cells in the Division of Immunology (Ref: I/871)

The salaries for the posts will be at the appropriate points on the Council's range equivalent to University non-clinical academic staff scales, plus £1,393 per annum London allowance and superannuation provision.

Applications should include a CV and a list of publications and the names of two professional referees. For the career appointment (Ref: V-871), a brief statement of the candidate's proposed research plans (about 1000 words) is also required. Applications should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA not later than Friday 3 April 1987 quoting the appropriate reference numbers. (2331)A

ST JOHN'S COLLEGE OXFORD AND THE CLARENDON LABORATORY

The College proposes to elect an Official Fellow and Tutor in Physics to take office on 1 October 1987. A titular University Lectureship in the Clarendon Laboratory is associated with this post. Further particulars may be obtained from the College Secretary, to whom applications should be sent by 17 April, 1987.

(2338)A

UNIVERSITY OF EDINBURGH
Edinburgh School of Agriculture
RESEARCH ASSISTANT ON ANIMAL BEHAVIOUR

Applications are invited for a post in the Animal Division to carry out research on Housing Systems for laying hens. Initial studies indicate that it is possible to modify conventional cages to improve the welfare of hens while retaining economic competitiveness. Funds are available from MAFF for two years for more detailed work on the behavioural response of hens to such modifications. Candidates should hold a relevant first degree and have adequate postgraduate experience. Starting salary will be £6,711 per annum.

Further particulars and application forms may be obtained from the Secretary. The Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG. Reference A8/Maff should be quoted in all communications. Closing date for applications: 30th March 1987. It is hoped that the appointment will start on 1 May 1987.

(2345)A

THE UNIVERSITY OF LAUSANNE

is inviting entries for filling the post of

Ordinary Lecturer in Mineralogy-Petrography

To take up the post: 1st September 1988. The holder will take on part of the basic teaching of the Institute of Mineralogy and Petrography (IMP).

Preference will be given to a geologist or mineralogist inclining towards geochemistry (with a specialization in petrology, stratology or similar) and interested in conducting research which backs up or complements that conducted by the Institute.

Applications (with c.v., list of publications, abstracts from most significant publications, research projects undertaken and references) may be sent until 30th September 1987 to the **Doyen de la Faculte des Sciences, collège propédeutique, CH-1015 Lausanne.**

Further information may be obtained from Prof. A. Steck, IMP, Palais de la Rumine, 1005 Lausanne. (W3893)A

UNIVERSITY OF OXFORD

Department of Pharmacology

POSTDOCTORAL ELECTROPHYSIOLOGIST/ NEUROPHARMACOLOGIST

Applications are invited for a research assistant (Grade 1A) tenable from the 1st October 1987 for three years. The post, financed by the Parkinson's Disease Society entails assisting on a multidisciplinary project concerned with the significance of secretory neuropeptides in CNS degeneration. Applicants should have experience of intracellular recording in brain slices *in vitro* and an interest in neuropharmacology. Further details can be obtained from **Dr S.A. Greenfield, University Department of Pharmacology, South Parks Road, Oxford OX1 3QT**. Applications (curriculum vitae, publications and names of three referees) should be sent to Dr Greenfield by 1st May 1987. (2303)A

MEDICAL RESEARCH COUNCIL

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

Division of Physical Biochemistry

Applications are invited for a career appointment to conduct research in molecular enzymology. Interest in the mechanisms of nucleoside triphosphatases involved in biological energy transduction and the regulation of cellular processes would be an advantage. The successful candidate will be expected to pursue his/her own research programme, and ample opportunity will be available for collaboration within the Institute. The Division is part of the Physiological and Neural Mechanisms Group at NIMR.

The salary will be at an appropriate point on the Council's range equivalent to University non-clinical academic staff scales, plus £1,393 London allowance per annum and superannuation provision.

Applications including a curriculum vitae, list of publications, a brief statement of research plans (about 1000 words), and the names of two professional referees, should be sent to the Director, National Institute for Medical Research, Mill Hill, NW7 1AA, not later than 3rd April, 1987 quoting Ref: PB/871W. (2317)A

ASTON UNIVERSITY

DIVISION OF BIOLOGY — DEPARTMENT OF MOLECULAR SCIENCES

CONTRACT RESEARCH ASSISTANT

Applications are invited for the above appointment to work with Dr P. J. Hanson on a project funded by the Wellcome Trust on the regulation of mucus secretion by isolated gastric mucosal cells. The overall aim of the work is to advance understanding of mechanisms responsible for protecting the gastric epithelium from damage by acid and pepsin.

Applicants should possess, or expect to attain, an honours degree of class 1 or 2(i) in biochemistry, cellular physiology or a related discipline. The post is available from September 1987 for 2 years in the first instance with the possibility of extension for a further period. The commencing salary will be £7,055 per annum (presently under review). The successful candidate would be expected to register for a higher degree.

Application forms and further particulars may be obtained from the Personnel Officer (Academic Staff, (quoting Ref: 876/6), Aston University, Aston Triangle, Birmingham B4 7ET. Tel: 021 359 0870 (24-hour answerphone). Closing date for the receipt of applications is 3 April 1987. (2307)A

— *POSITION OPEN* —

IMMUNOLOGIST/GENETICIST

Small, newly established company engaged in biomedical laboratory services and manufacture of diagnostic reagents is seeking a PhD with training in genetics and immunology to guide laboratory operations, review and approve test results, and direct R&D activities. Consultation readily available from internationally recognized scientists affiliated with company. Midwest location. Competitive salary and opportunity to share in future growth of company. Send curriculum vitae and other information to: **Box NW635, *NATURE* Classified, 65 Bleecker Street, New York, NY 10012-2467.** *An Equal Opportunity Employer.* (NW635)A

THE UNIVERSITY OF MÜNSTER

Institute of Physiological Chemistry and Pathobiochemistry seeks:

C3 – Professor

The institute is seeking candidates with MD or PhD and postdoctoral experience able to establish vigorous research and teach medical biochemistry in German language. Inquiries are welcome from individuals interested in medically relevant cell biology problems and qualified to use methods of DNA cloning and sequencing and protein analysis. Applications including names of references and copies of the most significant publications may be sent within 6 weeks to: **Dekan der Medizinischen Fakultät, Prof. H. Schröer, Domegkstraße 3, D-4400 Münster, FRG.** (W3884)A

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the above post to work on a SERC funded project (duration 3 years) on fatty acid synthesis in oil-seed rape. Applicants should have a PhD (or expect to obtain one shortly) in Biochemistry, Botany or a related subject. Experience in lipid metabolism and/or protein purification would be useful but not essential. Salary range £8,020—£9,000 on the 1A research scale (under review).

Applications (2 copies) together with the names and addresses of two referees should be forwarded to the Establishment Officer, University College, P.O. Box 78, Cardiff CF1 1XL. Further particulars are available from Professor J. L. Harwood, Department of Biochemistry on 0222 874108. Closing date 2nd April 1987. Ref. 3203. (2324)A

UNITED MEDICAL AND DENTAL SCHOOLS

GUY'S CAMPUS

LABORATORY SCIENTIFIC OFFICER OR RESEARCH ASSISTANT

required to assist in a project on monoclonal antibodies to microbial and T cell antigens. Previous experience in immunology and/or cell culture desirable. Appointment is for two years in first instance; starting salary on Whitley Council scale or University Range IB (approximately £7,500 - £8,500 pa inclusive), according to qualifications and experience.

Applications, give full curriculum vitae and names of two referees, should be sent to the Staffing Officer, UMDS, St Thomas's Campus, Lambeth Bridge Road, London SE1 7EH, quoting Ref: G/IMM/290, by 26 March 1987. (2337)A

THE UNIVERSITY OF LEEDS

DEPARTMENT OF GENETICS

POSTDOCTORAL RESEARCH FELLOW

Applications are invited from postdoctoral molecular biologists or geneticists for the above post available for a fixed term of up to three years, funded by the SERC to characterize further recently cloned *nif* (nitrogen-fixation) genes of an *Agrobacterium* strain and search for similar genes from other strains.

Salary on the R/A 1A Range (£8,020—£12,780) (under review), according to age, qualifications and experience. Informal enquiries may be made to Dr G. R. K. Sastry (tel (0532) 431751 ext 598).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 50/20. Closing date for applications 2 April 1987. (2302)A

UNIVERSITY OF DUNDEE

DEPARTMENT OF BIOLOGICAL SCIENCES

LECTURESHIP IN GENETICS

Applications are invited from outstanding candidates in any area of molecular genetics, particularly including the genetics of eukaryotic cells. The starting date and salary are negotiable, the latter to be within the non-clinical lecturers' scale £8,020—£15,700 (award pending).

Applications with c.v. (4 copies) and the names of three referees should be sent to the Personnel Office, The University, Dundee, DD1 4HN. Please quote reference EST/6/87/H. Closing date 9th April 1987.

Informal enquiries to Dr G. A. Codd (Tel: (0382) 23181 Ext. 4767). (2347)A

VISITING INVESTIGATOR

A vacancy exists for an **IMMUNOLOGIST** wishing to spend one or more years in a laboratory investigating the regulation of anti-tumor immunity as a model of the immune response to replicating antigens. Applications from MD's interested in gaining research experience, or established investigators wishing to spend a sabbatical year will be considered. The salary will be $25,000 plus health insurance, and Institute housing is available.

The Trudeau Institute is a private, not-for-profit, basic medical research institution located on the shore of Saranac Lake, and near Lake Placid, NY.

Send curriculum vitae and names of three references to: **Dr Robert J. North, Director, Trudeau Institute, Inc., P.O. Box 59, Saranac Lake, NY 12983.** (NW579)A

MAX-PLANCK-INSTITUT FÜR BIOCHEMIE

Applications are invited from physical biochemists or physicists to join a group working on the determination of low-resolution structures of protein complexes by electron microscopical image reconstruction. Facilities include a modern electron microscope with cryo-equipment and on-line image processing and several computers. Previous experience in electron microscopy or image reconstruction would be advantageous. A staff appointment (non-tenure track: about 2,400 DM) is available, initially for 1 year, and may be renewable. Applications should be sent to: **Dr H.J. Schramm, MPI für Biochemie, D-8033 Martinsried bei Munchen, West Germany.**

(W3911)A

ASSISTANTSHIPS

UNIVERSITY OF GLASGOW

APPLIED ORNITHOLOGY UNIT

RESEARCH ASSISTANT

Applications are invited for a research assistantship, funded by the Natural Environment Research Council, to study the nutritional demands of egg production in birds. The research assistant will be appointed for 3 years and applicants should have a degree or equivalent in a biological subject. An interest in the breeding of cage birds would be an advantage. Salary will be on Range 1B of the scales for Research and Analogous Staff.

Further details are available from Dr D. C. Houston, Applied Ornithology Unit, Department of Zoology, University of Glasgow, Glasgow G12 8QQ, to whom applications, together with a c.v. and the names of two referees should be sent by 10th April, 1987.

(2329)A

UNIVERSITY OF NOTTINGHAM

CANCER RESEARCH CAMPAIGN LABORATORY

Applications are invited for a Postgraduate Research Assistantship in the Cancer Research Campaign Laboratories. The project is to evaluate the effects of certain fatty acids on cancer cells, and will involve tissue culture. A degree in cell biology, biochemistry or a related subject is desirable. The appointment will be supported by a grant from the Gloria Miles Cancer Foundation and will be for one year in the first instance.

For further details please contact: Dr M J Embleton, Cancer Research Campaign Laboratories, University of Nottingham, Nottingham, NG7 2RD. (2335)P

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GRANTS & SCHOLARSHIPS

DEE CORPORATION RESEARCH SCHOLARSHIPS

New Hall invites applications from well-qualified women for a Dee Corporation Scholarship for research. Any U.K. student is eligible who will have graduated before 1 October 1987, and who intends to study for the degree of Ph.D. of the University of Cambridge. There are ten Scholarships of which **at least six are intended for science subjects**. The Scholarships are tenable at New Hall from 1 October 1987. The value of each Scholarship is £1,000 a year, and will normally be tenable for three years, subject to satisfactory academic progress. Tenure of the Scholarship is conditional upon acceptance by the Board of Graduate Studies to whom applications should be made, naming New Hall as college of first preference, on the Cambridge Intercollegiate Graduate Application Scheme (C.I.G.A.S.) application form (available from the Board of Graduate Studies, 4 Mill Lane, Cambridge, CB2 1RZ). There is no separate application form other than that submitted to the board of Graduate Studies, but candidates who wish to be considered for this award are advised to submit their application as early as possible. Candidates should also write to inform the Graduate Tutor, New Hall, Cambridge, CB3 0DF of their application no later than 30 April 1987. (2350)H

THE UNIVERSITY OF SYDNEY

RICHARD CLAUDE MANKIN SCHOLARSHIP

WATER CONSERVATION (POSTDOCTORAL)

Applications are invited for the Richard Claude Mankin Scholarship from persons who hold the degree of doctor or Philosophy or who possess such equivalent standing approved by the University of Sydney. The object of the scholarship is to promote and encourage research at the University of Sydney related to water conservation. The scholarship is currently valued at $A25,414—$26,112 per annum plus a travel grant for the successful applicant if travelling to Australia from overseas. The award shall be tenable for one year in the first instance but, subject to satisfactory progress, may be renewed for a second year.

The University of Sydney reserves the right not to proceed with the award of this scholarship for financial or other reasons. Application forms and further information may be obtained from the Registrar (Attention: Scholarships Office), University of Sydney, NSW 2006, Australia, with whom applications close on 3 April 1987. (W3894)G

STUDENTSHIPS

UNIVERSITY OF EAST ANGLIA

Norwich

ORGANOMETALLICS IN ORGANIC SYNTHESIS

SERC CASE studentship with Beecham Pharmaceuticals. Uses of chiral organometallic π-complexes as intermediates for the enantiomer synthesis of the alkaloid dihydrodioscorine. Enquiries should be directed to Dr GR Stephenson, School of Chemical Sciences, University of East Anglia, Norwich NR4 7TJ. (2352)F

UNIVERSITY OF GLASGOW

Department of Biochemistry

POSTGRADUATE RESEARCH STUDENTSHIPS

Applications are invited as soon as possible for postgraduate studentships tenable from 1st October, 1987 to work in the following research groups:

Molecular Pharmacology, Biochemical Immunology and Parasitology, Enzymology and Protein Engineering, Control of Gene Expression, Biochemical Endocrinology, Plant Biochemistry and Molecular Biology, Membrane Biotechnology, Cardiac and Vascular Biochemistry, Neurobiochemistry.

Some of the projects that are available will involve collaboration with clinical departments and others will have strong industrial connections. The Department has excellent facilities for a very wide range of types of research.

Further information may be obtained from Professor CA Fewson, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ. Telephone: 041-339 8855, Ext 5266. (2359)F

continued on page 16

NEW HALL RESEARCH STUDENTSHIP

The College invites applications from suitably qualified women students in any subject for a Research Studentship tenable from 1 October 1987. The Award will be made for one year in the first instance, and will be renewable for a maximum of two further years. The value of the Studentship will be finally dependent upon the income available to the successful candidate from other sources, but the maximum award will be the equivalent of a State Studentship for research (currently £2,756) in addition to approved University and College fees.

The purpose of the Studentship is to enable the successful candidate to pursue as a member of New Hall a course of study in the University of Cambridge leading to the degree of PhD, MLitt, MSc, or MPhil. Tenure of the Studentship is condition upon acceptance by the Board of Graduate Studies to whom applications should be made, naming New Hall as college of first preference, on the Cambridge Intercollegiate Graduate Application Scheme (C.I.G.A.S.) application form (available from the Board of Graduate Studies, 4 Mill Lane, Cambridge CB2 1RZ). The award is open to students from the United Kingdom or from overseas. There is no separate application form other than that submitted to the Board of Graduate Studies, but candidates who wish to be considered for this award are advised to submit their application as early as possible. Candidates should also write to inform the Graduate Tutor, New Hall, Cambridge, CB3 0DF no later than 30 April 1987, giving details where appropriate of their likely financial needs and resources.

(2349)F

AFRC INSTITUTE OF ANIMAL PHYSIOLOGY AND GENETICS RESEARCH EDINBURGH RESEARCH STATION

AFRC Research Studentship Molecular Biology/ Biochemistry

Applications are invited for a studentship commencing in October 1987, to investigate gene expression in transgenic animals. The successful candidate will work with a group performing gene transfer in farm and laboratory animals to study the effects of gene dosage of a milk protein gene on mammary gland function in transgenic mice. The student will be required to register for a Ph.D degree at the University of Edinburgh.

Candidates should have or expect to have a 1 or 2.1 degree in Molecular Biology, Biochemistry, Genetics or a related subject.

Applicants should send a Curriculum Vitae and the names of two referees to: The Secretary, AFRC Physiology and Genetics Research Station, Roslin, Nr Edinburgh, Midlothian, EH25 9PS.

Informal enquiries to Dr AJ Clark, Tel 031-667 6901 Ext 64.

(2342)F

UNIVERSITY OF LEICESTER

DEPARTMENT OF BIOCHEMISTRY

PhD STUDENTSHIPS

tenable from October 1987, for candidates who have or will have a first or upper second class Honours degree in Biochemistry or related subjects, are available in the following areas:

1. Microbial Physiology and Biochemistry.
2. Medical and Cellular Biochemistry.
3. Structure, Organisation and Expression of Genes.
4. Macromolecular Interactions.
5. Biophysical Analysis of Macromolecules and Protein Engineering.

Detailed information on the projects available can be obtained from The Departmental Secretary, Mrs J. M. Dean, (0533) 523470. Applications should include a full c.v., names and addresses of two referees and be sent as soon as possible to Mrs Dean, Department of Biochemistry, University of Leicester, University Road, Leicester LE1 7RH.

(2312)F

LUDWIG INSTITUTE FOR CANCER RESEARCH

Cambridge Branch

PhD STUDENTSHIP

Applications are invited for a PhD studentship available for three years from October 1987 to work on oncogenes and anti-oncogenes in human cancer.

Applicants should have a first or upper second class honours degree in a biological or related subject.

Applications including up-to-date c.v. together with the names of two referees should be sent to: **The Administrator, Ludwig Institute for Cancer Research, MRC Centre, Hills Road, Cambridge CB2 2QH.**

(2330)F

INSTITUTE OF ANIMAL PHYSIOLOGY & GENETICS RESEARCH, CAMBRIDGE

AFRC

AFRC PhD STUDENTSHIP

Applications are invited for a research studentship to start in October 1987. The project will examine environmental influences on thyroid hormone receptors, sodium pump sites and mitochondrial function in relation to cellular metabolism and growth. New image processing and microfluorometric facilities will be used to develop assessment on mitochondrial function in undisrupted tissues.

The Institute provides an excellent academic environment for research training with first-class research facilities and currently has 30 registered PhD students.

Applicants should hold or expect a 1st or 2nd class Honours Science, medicine or veterinary medicine degree. Applications, including a full curriculum vitae and names of two referees, should be submitted to: **Dr R. J. Bicknell, Tutor for Research Students, AFRC Institute of Animal Physiology and Genetics Research, Babraham, Cambridge, CB2 4AT**, from whom further information can be obtained (Quote ref. RS87/1). Closing date 3rd April 1987.

(2320)F

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF PHYSIOLOGY

NeuroTech Research Unit

Applications are invited for a

RESEARCH STUDENTSHIP

tenable from October 1987. Research will be concerned with neuromuscular rehabilitation in man. Experience in computation will be required. Applicants should have, or expect to have, an Honours Degree in Physiology (including Human Physiology), Exercise Physiology, Movement Science, Sports Science or Physiotherapy.

Applications, together with the names of two referees, should be received not later than 1st July 1987, by Dr G. L. Kidd, Department of Physiology, The University, P.O.Box 147, Liverpool, L69 3BX, Tel. 051-709 6022 Ext. 2448.

(2308)F

PLANT BREEDING INSTITUTE

AFRC RESEARCH STUDENTSHIP

Applications are invited for a AFRC Research Studentship te able for three years from Septen ber/October 1987 for studies in th area of probe development for r striction fragment length pol morphisms (RFLPs) analysis wheat.

Candidates should possess expect to gain a good Honou degree in a relevant disciplin Applications with c.v. and th names and addresses of three r ferees should be sent to the Pe sonnel Officer, Plant Breedin Institute, Maris Lane, Trumpin ton, Cambridge CB2 2LQ, fro whom further particulars may obtained, by 3rd April 198 Please quote reference AFRC RS.

(2313)F

FELLOWSHIPS

UNIVERSITY OF SOUTHAMPTON

POSTDOCTORAL FELLOWSHIP IN PHOTOELECTRON SPECTROSCOPY OF SHORT-LIVED MOLECULES

Applications are invited for a postdoctoral fellowship to work with Dr J M Dyke on the use of multidetector photoelectron spectroscopy to study sthort-lived molecules in the gas phase. The post is available from 1 October 1987 but an earlier appointment could be considered. The initial appointment is for one year. As part of the project will be directed towards the study of silicon CVD processes, experience in preparature chemistry or spectroscopy would be desirable but not essential.

Starting salary within the £8.020 - £12,780 per annum (pay award pending), depends on age and experience. Applications, enclosing a curriculum vitae and the names of two referees should be sent to: **Dr. J. M. Dyke, Department of Chemistry, The University, Southampton SO9 5NH.** Please quote Ref No: 115/HFW/MB/NA.

(2336)E

continued on pages 17 & 1

POSTDOCTORAL FELLOWSHIP

A vacancy exists for a postdoctoral fellow interested in studying the regulation of immunity to tumour transplantation antigens and tumor histocompatibility antigens. Applicants should have a basic training in immunology. Starting salary will be $18,500 plus liberal fringe benefits. Subsidized Institute housing is available.

The Trudeau Institute is a private, not-for-profit, basic medical research institution located on the shore of Saranac Lake and near Lake Placid, NY.

Send curriculum vitae and names of three references to: Dr Robert J North, Director, Trudeau Institute, Inc., P.O. Box 59, Saranac Lake, NY 12983.

(NW578)E

UNIVERSITY OF EDINBURGH

DEPARTMENT OF FORESTRY & NATURAL RESOURCES

POSTDOCTORAL RESEARCH FELLOWSHIP

cophysiology of Tropical Trees

year appointment funded by
ERC to start in October 1987.
ie project will involve measure-
ents of gas exchange using por-
ble equipment in secondary
opical forest in Nigeria, and laboratory experiments using controlled environment chambers in Edinburgh. Principal Investigator Dr J. Grace.

Please send applications to arrive no later than 6 April to the Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh, EH1 1LS, enclosing c.v. and giving names of two academic referees. Salary on 1A scale £8,020—£12,780 (under review). Please quote reference No. 5448.

(2316)A

UNIVERSITY COLLEGE OF WALES ABERYSTWYTH

DEPARTMENT OF BOTANY & MICROBIOLOGY

Applications are invited from suitably qualified persons for the following posts which are available now or to start at a mutually convenient time:

Postdoctoral Research Fellowship on "The Exploitation of Electromicrobial Techniques in Biotransformations". This will involve the use of high electric fields selectively to permeabilise bacterial membranes and of bioelectrochemical techniques to drive stereoselective reductions of xenobiotics.

CASE PhD Studentship in collaboration with CAMR, Porton Down (Dr David J. Clarke) on "Survival and Metabolic Behaviour of Bacteria in Microemulsions". The work will characterise, principally by laser and dielectric methods, a novel class of water-in-oil microemulsions and will study the effects of these upon the viability of metabolic behaviour of selected bacteria incorporated therein.

Applications, containing a c.v., should be sent to **Dr Douglas Kell, Department of Botany & Microbiology, University College of Wales, Aberystwyth, Dyfed SY23 3DA, U.K.** Informal enquiries may be made by telephoning 0970 3111 ext 3055, or ext 3060.

(2310)A

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nature

— the professionals' choice

UNIVERSITY OF BIRMINGHAM FACULTY OF SCIENCE

1. Department of Physics and Chemistry (ref D4510/2).

Applications are invited for a up to three year post-doctoral Research Fellowship to take part in a Neutron Scattering research programme on the structures and diffusion of liquids in 2-dimensional and Fractal Silicate Systems using the pulsed neutron source ISIS at the Rutherford Appleton Laboratory and the High Flux Reactor at the Institute Laue — Langevin at Grenoble.

2. Department of Biochemistry (ref B4530).

Applications are invited for a up to two year post-doctoral Research Fellowship, which is funded by the Parkinson's Disease Society. The project will involve investigating in the laboratory of Dr RH Waring pathways of Drug metabolism in patients with Parkinson's Disease. Experience of toxicology or clinical chemistry would be an advantage.

Both appointments on the Research Fellow 1A scale £8,020 — £12,780 (under review) plus superannuation. For further particulars for each of these two posts, telephone 021-472 1301 ext 2559, quoting reference numbers as stated above. No formal application. Three copies of application, including full Curriculum Vitae and naming three referees to Assistant Registrar (Science), PO Box 363, Birmingham, B15 2TT by 3 April 1987.

An equal opportunities employer.

(2343)E

Research Fellowships

Post-doctoral Fellowships are available in the Fund's laboratories for a number of research projects. The awards will be tenable for three years with a salary range of £9,830 to £12,205 plus £1,465 Inner London Weighting or Outer London Weighting £977 a year. Entry depending on qualifications and experience. Appointments are available in the following fields:

1. **HLA studies — molecular manipulation of HLA genes.**
   JULIA BODMER
2. **Molecular genetics of colorectal carcinomas.**
   SIR WALTER BODMER
3. **Comparison of the biological activities of human papilloma viruses types 6 and 16.**
   LIONEL CRAWFORD
4. **Interaction of growth factors in the steroid hormone regulation of breast cancer cells in tissue culture.**
   PHILLIPA DARBRE
5. **Regulation of human β interferon gene.**
   STEPHEN GOODBOURN
6. **Generation of monoclonal antibodies to melanoma lines of differing metastatic activity.**
   IAN HART
7. **Mitogenic control and second messengers in metastatic tumour variants.**
   IAN HART
8. **Molecular analysis of human macrophage genes.**
   NANCY HOGG
9. **Molecular analysis of bone cell specific genes.**
   MICHAEL HORTON
10. **Molecular cloning of a mutator gene from hamster cells.**
    MARK MEUTH (Clare Hall)
11. **T Cell receptor alpha and beta variable region usage in autoimmune T lymphocytes.**
    MICHAEL OWEN
12. **MHC-T cell receptor interaction at the molecular level studied by DNA-mediated gene transfer.**
    MICHAEL OWEN (with John Trowscale)
13. **Molecular aspects of steroid hormone action.**
    MALCOLM PARKER
14. **Mucin genes and carcinomas.**
    JOYCE TAYLOR-PAPADIMITRIOU
15. **Gene expression in osteoid tissue.**
    NATALIE TEICH
16. **Optimisation of antibody-toxin conjugates (immunotoxins) for therapy of solid tumours.**
    PHILIP THORPE
17. **Immunotoxins for the selective destruction of tumour vasculature.**
    PHILIP THORPE
18. **Gene function and regulation in the human histocompatibility complex.**
    JOHN TROWSDALE
19. **Molecular analysis of human alkaline phosphatases and other potential tumour markers.**
    DEREK TUCKER
20. **Molecular basis of keratinocyte — substratum adhesion.**
    FIONA WATT
21. **Isolation of recombination enzymes from human cells.**
    STEPHEN WEST (Clare Hall)
22. **Function of human cytochrome P-450's in carcinogen activation.**
    ROLAND WOLF (Edinburgh)

Further details are available from the Personnel Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX on 01-242 0200 ext 2305. Quoting reference 75/87/R. Applications should be accompanied by the names and addresses of three referees and be received no later than 27 March, 1987

(2323)E

IMPERIAL
CANCER RESEARCH FUND

MALAGHAN INSTITUTE OF MEDICAL RESEARCH

Wellington School of Medicine
Wellington Hospital
Wellington 2, New Zealand

BRIERLEY SENIOR MEDICAL RESEARCH FELLOW

Applications are invited from medical and non-medical graduates for the position of Brierley Senior Medical Research Fellow. The successful applicant will engage in full-time research into either some field of cancer, heart disease or stroke — preferably to be compatible with and to complement ongoing research programmes in the Institute. The position is for five years in the first instance with the possibility of renewal.

Salary: Senior Medically Qualified Research Fellow $56,808—$71,712. Senior Non-Medically Qualified Research Fellow $40,000—$63,660.

Intending applicants are invited to write for further particulars from the Director, Professor E.W. Stehbens, Malaghan Institute of Medical Research, Wellington School of Medicine, Wellington Hospital, Wellington 2, New Zealand.

Applications close in Wellington on April 30, 1987. (W3898)E

THE AUSTRALIAN NATIONAL UNIVERSITY

invites applications from suitably qualified men and women for

POSTDOCTORAL OR RESEARCH FELLOWSHIPS IN NUCLEAR PHYSICS

The Department of Nuclear Physics (Head: Professor J. O. Newton, FAA), Research School of Physical Sciences, conducts research in nuclear structure physics. The purpose of the work is to gain greater understanding of both the static and dynamic aspects of the isolated atomic nucleus and of its interactions with other nuclei when collisions occur. Currently, the principal areas of research are: reactions with heavy ions, including resonant structure; measurements of static quadrupole moments of excited states through Coulomb excitation; nuclei with very high angular momenta studied through gamma, particle and fission decay; production of exotic nuclei far from the stability line. In addition work is carried out on the interaction of charged particle beams with solids; investigations of energy loss and the optical and X-ray spectroscopy of highly charged ions are made. Closing date: 30 April 1987. Ref: PS 21.1.1.

Research Fellow A$27,859—$36,600 p.a.; Postdoctoral Fellow Grade 1 A$24,013—$27,507 p.a. Grants towards travel, assistance with housing, superannuation are available. Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time. Applicants should quote the reference number when submitting applications to R. V. Dubs, Registrar, GPO Box 4, Canberra, ACT, 2601, Australia.

The University is an equal opportunity employer.

(W3895)E

UNIVERSITY OF OTAGO

Dunedin, New Zealand

POSTDOCTORAL FELLOWSHIP IN CHEMISTRY

Applications are invited from suitably qualified candidates for a U.G.C. Postdoctoral Fellowship to carry out research of importance to the development of New Zealand's petrochemical industry. The project title is "Electrocatalytic synthesis using organometallic clusters" and the successful applicant will be part of a research team led by Professor Brian Robinson and Associate Professor Jim Simpson of the University of Otago Department of Chemistry. Experience in electrochemistry or synthetic chemistry would be an advantage, as the work involves the application of electron transfer chain analysis to petrochemical synthesis. Further information may be obtained from Professor Robinson in the University (tel: 771-640 ext. 526).

The Fellowship, tenable immediately, will be awarded for two years, and applicants should have completed the requirements for the award of the degree of Doctor of Philosophy. Salary: NZ$30,500 per annum.

Applications, quoting reference number A87/7, and including a detailed c.v. and names of two referees, should be sent as soon as possible to the Registrar, University of Otago, P.O. Box 56, Dunedin, New Zealand. A copy should also be sent to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom further particulars are available.

(W3907)E

SEMINARS & SYMPOSIA

NATO/EMBO/FEBS

International Summer School on Molecular and Cell Biology

The Spetsai Summer School, 1987, sponsored by NATO, EMBO and FEBS will be held in Spetsai, Greece, from August 30th to September 12th, 1987. The subject will be "Molecular Biology of Development". Lectures, discussions, and tutorials in English will cover the following topic areas:

1. **Cell Lineage and Cell Fate**
2. **Developmental Genes**
3. **Somatic Differentiation**
4. **Tissue-specific Gene Regulation**
5. **Plant Development**

The school will be suitable for advanced predoctoral students and for postdoctoral workers in related fields. Lecturers will include:

S. Artavanis-Tsakonas (USA)
D. Baltimore (USA)
T. Caskey (USA)
V. Chapman (USA)
A. Evangelopoulos (Greece)
W. Gehring (CH)
H. Goodman (USA)
P. Gruss (W Germany)
J. Hershey (USA)
R. Jaenisch (USA)
A. McLaren (UK)
G. Morata (Spain)
P. Overbeek (USA)
R. Palmiter (USA)
R. Phillips (Canada)
J. Sulston (UK)
W. Schaffner (CH)
J. Schell (W Germany)
E. Wagner (W Germany)
D. Wolgemuth (USA)

Applications should be sent to: The Spetsai Summer School Secretary, Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030 USA, no later than April 27, 1987. They should contain a curriculum vitae, including the applicant's scientific background and interests, plus a letter of recommendation. The applicants will be informed by May 25, 1987, whether they have been accepted. A deposit of US $100 will then be due. In general, participants will be required to pay for demi-pension accommodation (an extra US $100) and their travel expenses. A limited number of fellowships are available towards the cost of travel and accommodation in special cases.

(NW625)M

INTERNATIONAL SYMPOSIUM ON PLANT TRANSPOSABLE ELEMENTS

22—26 August 1987
University of Wisconsin
Madison, Wisconsin

The program (with 35 speakers) is designed to present an integrated view of plant transposable elements from the formal genetics to the most recent research. There will be an emphasis on the molecular aspects such as structure and effects on splicing of the primary transcript. The various strategies to utilize transposon tagging to identify the clones of interesting genes will be explored in detail. Posters are invited. For further information and a registration brochure which includes the program, contact: **Transposable Element Symposium, Wisconsin Center, 702 Langdon Street, Madison, WI 53706. Telephone: (608) 262-5514.**

Registration deadline is August 1, 1987. Attendance will be limited to 200. (NW617)M

continued on pages 19 & 2

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First CBT (Center for Biotechnology) Minisymposium on:
NUCLEAR ONCOPROTEINS: ROLES IN INDUCTION AND MAINTENANCE OF NEOPLASIA

STOCKHOLM
June 12-13, 1987

Organizing Committee:
Rolf Ohlsson Rune Toftgard Georg Klein

Programme:
- ☐ Control of gene expression
- ☐ Hormone receptors
- ☐ Molecular basis for oncoprotein functions
- ☐ Cell cycle progression
- ☐ Cellular maturation
- ☐ Cellular transformation
- ☐ Transgenic mice

Invited participants:
J. Adams *(Viktoria)*
F. Alt *(New York)*
M. Cole *(Princeton)*
R. Eisenman *(Seattle)*
T. Graf *(Heidelberg)*
P. Leder *(Boston)*
R. Muller *(Heidelberg)*
K. Nilsson *(Uppsala)*
U. Rapp *(Frederick)*
R. Roeder *(New York)*
G. Evan *(Cambridge)*
B. Venstrom *(Heidelberg)*
E. Ziff *(New York)*
G. Akusjarvi *(Uppsala)*
P. Chambon *(Strasbourg)*
B. DeChrombrugghe *(Bethesda)*
T. Gonda *(Viktoria)*
M. Groudine *(Seattle)*
K. Marcu *(New York)*
A. Nienhuis *(Bethesda)*
M. Oren *(Rehovot)*
E.P. Reddy *(Philadelphia)*
D. Stehelin *(Lille)*
R. Watt *(Philadelphia)*
I.M. Verma *(San Diego)*

Applications:
A letter of application, containing a brief outline of research interests and ambitions, should be sent to the address below before March 15, 1987. The total number of participants will be limited to 125.

Rolf Ohlsson, Center for Biotechnology, Karolinska Institute, Huddinge University Hospital F82, S-141 86 Huddinge, Sweden
(W3892)M

BAYLISS & STARLING SOCIETY
ANNUAL SCIENTIFIC MEETING
28th-29th SEPTEMBER 1987
THE QUEEN'S UNIVERSITY OF BELFAST BELFAST, NORTHERN IRELAND

A two-day programme of free communications and posters on the physiology, biochemistry, pathology and phylogenetic aspects of

Regulatory Peptides in the Brain and in the Gastrointestinal Tract

The programme will include a symposium on:

"Gastrointestinal Apudomas"

Invited speakers include
Professor Stephen Bloom, Dr Michael Conlon, Dr Larry Kvols, Professor Julia Polak and Professor Flemming Stadil

The meeting will also include a guest lecture by Dr Kazuhino Tatemoto on "Chemical and biological properties of recently-discovered peptide hormones"

For further details including Abstract forms contact:
Professor Keith D. Buchanan
Department of Medicine
Institute of Clinical Science
Grosvenor Road, Belfast BT12 6BJ
Tel: 0232-240503 Ext. 2585

Deadline for Abstracts: 17 July 1987

The Bayliss and Starling Society is a Registered Charity (No. 279143) (2309)M

National Institutes of Health announces an

Alumni Reunion and Program of Scientific Symposia

October 15 - 18, 1987

This event will be the culminating program of the NIH Centennial year (1887 - 1987). If you have ever been a part of the NIH, you are cordially invited to attend any or all of the following events:

October 15 - 16	**Scientific Symposia** Bethesda, Maryland
October 17	**Plenary Symposium** Kennedy Center Washington, D.C. **Banquet and Dance** Pension Building Washington, D.C.
October 18	**Brunch** Bethesda, Maryland

To receive your invitation and information on registration and fees, please write or call:

Ms. Andrea Manning
NIH Centennial
c/o Prospect Associates
1801 Rockville Pike, Suite 500
Rockville, Maryland 20852
301/468-6555 (NW610)M

INTERNATIONAL SYMPOSIUM ON:

STRUCTURE, FUNCTION AND REGULATION OF MEMBRANE PROTEINS

Hotel Fürigen, Stansstad (NW), Switzerland
August 2-6, 1987

The general topics to be considered include synthesis and expression of transport systems, structure/function of membrane transport systems, Na cotransporters, exchangers and channels, and regulation of membrane transport systems. The format will include invited state of the art presentations in the mornings, posters and poster discussions in the late afternoons, and special keynote lectures in the evening.

Invited speakers include: Q. Al-Awquati (New York); W. Boron (New Haven); E. J. Bowman (Santa Cruz); E. Carafoli (Zurich); R. Farley (Los Angeles); K. Geering (Lausanne); G. Giebisch (New Haven); R. Greger (Freiburg); S. Grinstein (Toronto); P. Jörgensen (Aarhus); R. Kaback (Nutley); J. P. Krähenbühl (Lausanne); M. Ladzunski (Nice); H. Lodish (Boston); D. Louvard (Paris); D. Meyer (Heidelberg); W. Molenar (Utrecht); H. Murer (Zurich); J. Pouyssegur (Nice); B. Rossier (Lausanne); G. Sachs (Los Angeles); G. Semenza (Zurich); G. Shull (Cincinnati); K. Simons (Heidelberg); K. Spring (Bethesda); K. J. Ullrich (Frankfurt); D. Warnock (San Francisco); E. Wright (Los Angeles).

For further information about the meeting and preliminary applications: Dr Heini Murer, Physiologisches Institut, Universität Zürich, Winterhurerstrasse 190, CH-8057 Zurich, Switzerland.
(W3887)M

INTERNATIONAL SUMMER SCHOOL
on the
MOLECULAR GENETICS OF DIFFERENTIATION

Berlin (West) 12 – 24 July 1987

ORGANIZERS: V. E. A. RUSSO, Berlin
T. LEIGHTON, Berkeley

ADVISORY COMMITTEE:
D. J. COVE, Leeds
A. GARCIA-BELLIDO, Madrid
A. KLAR, Cold Spring Harbor

SCIENTIFIC PROGRAM

Bacillus	T. LEIGHTON, Berkeley
	R. LOSICK, Cambridge, MA
Myxococcus	D. KAISER, Stanford
Aspergillus	C. SCAZZOCCHIO, Orsay
	W. E. TIMBERLAKE, Davis
Dictyostelium	G. GERISCH, Martinsried
	W. F. LOOMIS, La Jolla
Neurospora	V. E. A. RUSSO, Berlin
Yeast	A.KLAR, Cold Spring Harbor
	K. NASMYTH, Cambridge, GB
Agrobacterium-Plant	J. SCHELL, Köln
	L. WILLMITZER, Berlin
Maize	P. STARLINGER, Köln
Moss	D. J. COVE, Leeds
Caenorhabditis	B. MEYER, Cambridge, MA
Trypanosomes	N. AGABIAN, Berkeley
Drosophila	A. GARCIA-BELLIDO, Madrid
	C. NÜSSLEIN-VOLHARD, Tübingen
Mouse	R. JAENISCH, Cambridge, MA
	E. F WAGNER, Heidelberg
Immune-System	L. HOOD, Pasadena
	F. MELCHERS, Basel
Steroid Hormones	G. SCHUTZ, Heidelberg
Oncogenes	K. BISTER, Berlin

APPLICATION

The school is open to applicants with Ph.D. degrees or equivalent. Applications of outstanding graduate students (or equivalent) will also be considered. Participation is strictly limited to 80 students. Applications should include a FULL c.v. and specific reasons for wishing to attend the school. A letter of recommendation may be added if such information is thought to be important in facilitating the selection process.

Applications should be sent to: **Dr. V. E. A. RUSSO, MAX-PLANCK-INSTITUT FÜR MOLEKULARE GENETIK, IHNESTRASSE 73, D-1000 BERLIN 33,** and should arrive no later than **21 April 1987.** Applicant will be informed no later than 1 May 1987 whether or not they were selected. There are no special application forms.

REGISTRATION FEE:
Registration fee is DM 1.000.For applicants from industrial corporations the registration fee is DM 2.500. This includes room and board. Registration fee will be paid upon notice of acceptance to the school. The school is residential and all participants will be accommodated in a conference center in the peaceful environment of Monsanto. Several fellowships for travel and/or room and board are available.

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FIRST ANNOUNCEMENT, CALL FOR POSTERS AND REGISTRATIONS

THIRD INTERNATIONAL CONFERENCE ON PROGRESS IN CANCER RESEARCH
SAN REMO, ITALY MAY 4–6, 1987

Organized by:

National Institute for Cancer Research — Genoa, Italy
Italian League Against Cancer

Scientific and Organizing Committee:

Renato Dulbecco, U.S.A. (Chairman)	Paul Marks, U.S.A.
Nikolai N. Blokhin, U.S.S.R.	Leonardo Santi, Italy
Vincent De Vita, U.S.A.	Luciano Zardi, Italy

General Secretary:

Giovanni Lotti, Italy

Conference topics will focus on:

Oncogenes — Growth Factors — Antioncogenes — Monoclonal Antibodies in Tumor Diagnosis and Therapy — Chemotherapy and Drug Resistance — Interferon Interleukin-2 and LAK Cells in Tumor Therapy — Reversion of the Malignant Phenotype — Cell-Cell Adhesion, Cell-Extracellular Matrix Adhesion and Metastasis — Tumor Stroma Generation

Invited speakers include:

Webster Cavenee, Canada; Luigi Chieco-Bianchi, Italy; Giuseppe Della Porta, Italy; Bernard Fisher, U.S.A.; Janice Gabrilove, U.S.A.; Judy Gasson, U.S.A.; Jordan Gutterman, U.S.A.; Henry Harris, England; Dan Longo, U.S.A.; Michael Lotze, U.S.A.; John Minna, U.S.A.; Rita Levi Montalcini Italy; Malcolm Moore, U.S.A.; Lorenzo Moretta, Italy; Pier Giorgio Natali, Italy; Kenneth Olden, U.S.A.; Ira Pastan, U.S.A.; Giorgio Prodi, Italy; Neal Rosen, U.S.A.; Jean Paul Thiery, France; Inder Verma, U.S.A.; Bengt Westermark, Sweden; Owen Witte, U.S.A.

Registration fee, including lunch, will be 350,000 Italian Liras (about 240 US Dollars). A limited number of fellowships will be available. Application form and abstract should be submitted before April 15, 1987 to:

M. Grazia Moro, Instituto Nazionale per la Ricerca sul Cancro, Viale Benedetto XV, 10, 16132 GENOVA, Italy. Tel. 39–10–352855 — Telex 216353 ISTEX 1 (W3882)C

ANNOUNCING THE

FOURTH EUROPEAN SEMINAR & EXHIBITION
ON
COMPUTER-AIDED MOLECULAR DESIGN
8th/9th October 1987, Helsingør, Denmark

This popular annual meeting will bring you up-to-date with the latest developments in computer graphics and chemistry. Session topics will include molecular modelling; drug design; protein structure; dynamics; NMR; the problems of solvation; protein engineering; new types of calculation and their application to nucleic acids, sugars and materials; computer-aided synthesis and the impact of artificial intelligence. In addition some of the leading companies involved in developing the hardware and software will be at the exhibition alongside.

Full details from: **Penny Robinson, IBC Technical Services Ltd, Bath House, 56 Holborn Viaduct, London EC1A 4EX. Tel 01-236 4080, Tlx 888870.** (W3912)M

CAPRI LECTURE IN BIOTECHNOLOGY

REGULATION OF MAMMALIAN CELL GROWTH

Capri (Italy) — Europa Palace Hotel Anacapri September 27 to October 2, 1987

Organized by: **CIBA-GEIGY and T&B Tecnologie per la Bioingegneria**

Main Topics:	**Speakers:**
Gene Expression	D. Baltimore (Cambridge, USA), I. Bozzoni (Rome), P. Sassone Corsi (San Diego), W. Schaffner (Zürich)
Oncogenes	W.W. Franke (Heidelberg), G.J. Todaro (Seattle), H.E. Varmus (San Francisco), I.M. Verma (San Diego)
Hormones	P. Chambon (Strasbourg), R.M. Evans (San Diego), W.J. Rutter (San Francisco)
Viruses	P. Amati (Rome), F. Cuzin (Nice), R. Dulbecco (San Diego), R.C. Mulligan (Cambridge, USA)
Lymphokines and Immune Response	D.V. Goeddel (San Francisco), T.H. Rabbitts (Cambridge, GB), C. Sorg (Münster), T.A. Waldmann (Bethesda)
Conference Summary	M.L. Birnstiel (Vienna).

APPLICATIONS:

Applicants should submit a curriculum vitae and a one-page abstract of their research activities (with title, author(s), affiliation, and text; typed camera-ready within a frame of 16×23 cm.). From these abstracts, 10-15 will be selected for a short oral presentation, and the remaining participants will be invited to present a poster.

Applicants will be selected by the Scientific Committee which includes: E. Borrelli (San Diego), P. Chambon (Strasbourg), H. Hofstetter (Basel), P. Sassone-Corsi (San Diego) and W. Schaffner (Zürich). Applicants will be informed by June 15 whether they have been accepted.

The applications should be addressed to:

Dr H. Hofstetter — Ciba-Geigy AG, Biotechnologie K-681, 4002 Basel, Switzerland. Telex: 962991 — Fax: + 41-61-377444.

The deadline for receipt of applications is May 10, 1987.

INFORMATION:

For general information please contact the Operating Secretariat: **T&B Tecnologie per la Bioingegneria** — Attn.: Maria Rosaria di Mauro, Via Tasso 428/A — 80127 Napoli — Italy. Tel. + 39-81-658033/659021 — Telex: 720573.

TECHNOLOGICAL EXHIBITION:

There will also be a technological exhibition of products, instrumentation and technologies related to the subjects covered by the conference. Interested companies should contact *T&B Tecnologie per la Bioingegneria* by April 30, 1987.

CONFERENCE REGISTRATION:

Registration fee is US$200. It includes documentation, admission to the scientific program, coffee breaks, social activities. Participants will be accommodated in hotels of various price classes. The registration fee does NOT cover the cost of accommodation. The total number of participants will be limited to 200.

(W3883)C

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(W3899)L

ANNOUNCEMENTS

Introducing the Journal of Liposome Research

CALL FOR PAPERS

The Journal of Liposome Research is a new publication by Marcel Dekker, Inc. whose mission is to present high quality original liposome research and a small number of selected reviews. The subjects will be broad, ranging from biophysical analysis of liposome membranes to clinical applications of liposome-encapsulated drugs. Only papers focused on some aspects of liposome research will be considered. Dr Marc J. Ostro, Vice Chairman and Chief Science Officer of The Lipsome Company, Inc. will be the editor-in-chief and to whom all manuscripts should be submitted. The Journal has attracted an outstanding international editorial board detailed below. It is anticipated that the first issue will be published in the Summer of 1987 and will initially appear quarterly. **EDITOR-IN-CHIEF, Marc J. Ostro, PhD, Vice Chairman and Chief Science Officer, The Liposome Company, Inc., One Research Way, Princeton, New Jersey 08540**

EDITORIAL BOARD:

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(NW611)G

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GUIDE TO AUTHORS

Please follow these guidelines so that your manuscript may be handled expeditiously.

Nature is an international journal covering all the sciences. Contributors should therefore bear in mind those readers for whom English is a second language and those who work in other fields. Please write clearly and simply, avoiding unnecessary technical terminology. *Nature*'s staff will edit manuscripts to those ends if necessary. Contributors should check their proofs carefully.

Because of the competition for space, many of the papers submitted for publication cannot be accepted. For this reason, and because brevity is a great assistance to readers, papers should be as brief as is consistent with intelligibility. Please note that one printed page of *Nature*, without diagrams or other interruptions of the text, has fewer than 1,300 words.

CATEGORIES OF PAPER

Review Articles survey recent developments in a field. Most are commissioned, but suggestions are welcome in the form of a one-page synopsis addressed to the Reviews Coordinator. Length is negotiable in advance but must not exceed six pages of *Nature*.

Articles are research reports whose conclusions are of general interest and which are sufficiently rounded to be a substantial advance in understanding. They should not have more than 3,000 words of text or more than six display items (figures and tables) and should not occupy more than five pages of *Nature*.

Articles should be accompanied by a heading of 50 – 80 words written to advertise their contents in general terms, to which editors will pay particular attention. A heading (printed in italic type) is not an abstract and should not usually contain numbers or measurements. The study should be introduced in more detail in the first two or three paragraphs, which should also briefly summarize its results and implications.

Articles may contain a few subheadings of two or three words. The meaning of the text should not depend on the subheadings, whose function is to break up the text and to point to what follows. There should be fewer than 50 references.

Letters to Nature are short reports of outstanding novel findings whose implications are general and important enough to be of interest to those outside the field. Letters should not have more than 1,000 words of text or more than four display items and should not occupy more than two pages of *Nature*. The first paragraph should describe, in not more than 150 words, the origins and chief conclusions of the study. Letters should not have subheadings or more than 30 references.

Commentary articles deal with issues in, or arising from, research that are also of interest to readers outside research. Some are commissioned, most are unsolicited. They are normally between one and four pages of *Nature* in length.

News and Views articles are intended to inform non-specialist readers about a recently published advance. Suggestions should be made to the News and Views Coordinator. Illustrations are welcome. Proposals for meeting reports should be agreed in advance.

Scientific Correspondence is for the discussion of scientific matters, including contributions published in *Nature*. Priority is given to contributions of less than 500 words and five references. Figures are welcome.

GENERAL

All contributions submitted for publication in *Nature* should conform with these rules.

Manuscripts should be typed, double-spaced, with a good-quality printer, on one side of the paper only. Four copies are required, each accompanied by lettered artwork. Four copies of half-tones should be included. Reference lists, figure legends and so on should be on separate sheets, all of which should be double-spaced and numbered. Copies of relevant manuscripts in press or submitted for publication elsewhere should be included, clearly marked as such. Revised and resubmitted manuscripts should also be clearly marked as such and labelled with their reference numbers.

Titles should say what the paper is about with the minimum of technical terminology and in fewer than 80 characters. Authors should avoid active verbs, numerical values, abbreviations and punctuation and should include one or two key words for indexing purposes.

Artwork should be marked individually and clearly with the author's name, figure number and, when known, manuscript reference number. Original artwork should be unlettered. Ideally, no figure should be larger than 28 by 22 cm. Figures with several parts are permitted only if the parts are closely related, either experimentally or logically. Suggestions for cover illustrations, with captions, are welcome. Original artwork (and one copy of the manuscript) will be returned when a manuscript cannot be published.

Figure legends must not exceed 300 words and ideally should be much shorter, and should use telegraphic form wherever possible. The figure should be described first, then, briefly, the method. Reference to a method described elsewhere is preferable to a full description. Methods should not be described in detail in the text.

References should be numbered sequentially as they appear in the text, followed by those in tables and finally those in the figure legends. Reference numbers apply only to papers published or in the press, and a different number should be given to each paper cited. All other forms of reference (including unrefereed abstracts) should be included in the text as personal communication, manuscript in preparation or preprint (with number and institution where appropriate). Text should not be included in the reference list. References should be abbreviated according to the *World List of Scientific Periodicals* (fourth edition, Butterworth, London, 1963 – 65). The first and last page numbers should be cited. Reference to books should clearly indicate the publisher and the date and place of publication.

Abbreviations, symbols, units and Greek letters should be identified the first time they are used. Acronyms should be avoided as much as possible and, when used, defined. Editors will shorten words if necessary. In case of doubt, SI units should be used.

Footnotes should not be used except for changed addresses or to identify the corresponding author if different from the first-named.

Acknowledgements should be brief. Grant numbers and contribution numbers are not allowed.

Submission. *All manuscripts may be submitted to either London or Washington. They should not be addressed to editors by name. Manuscripts or proofs sent by air courier to London should be declared as 'manuscripts' and 'value $5' to prevent the imposition of import duty and value-added tax (VAT).*

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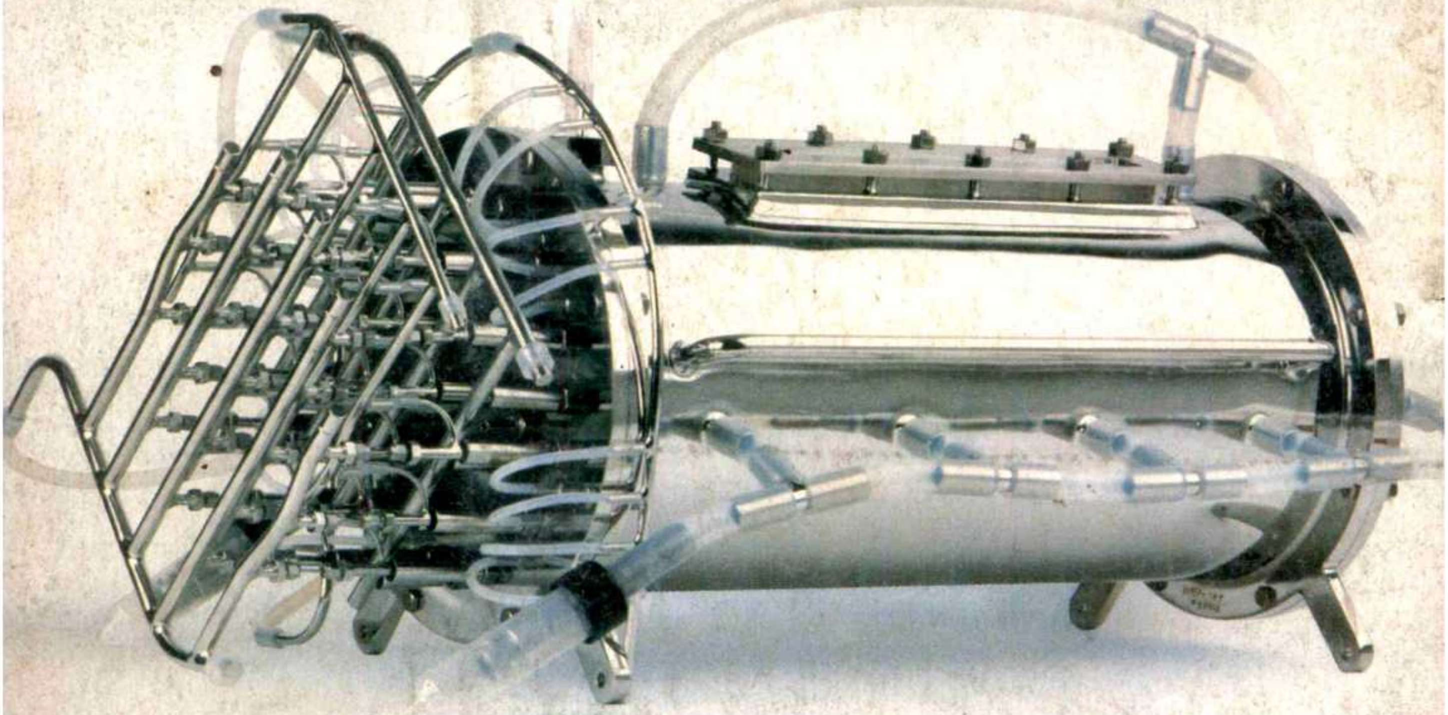

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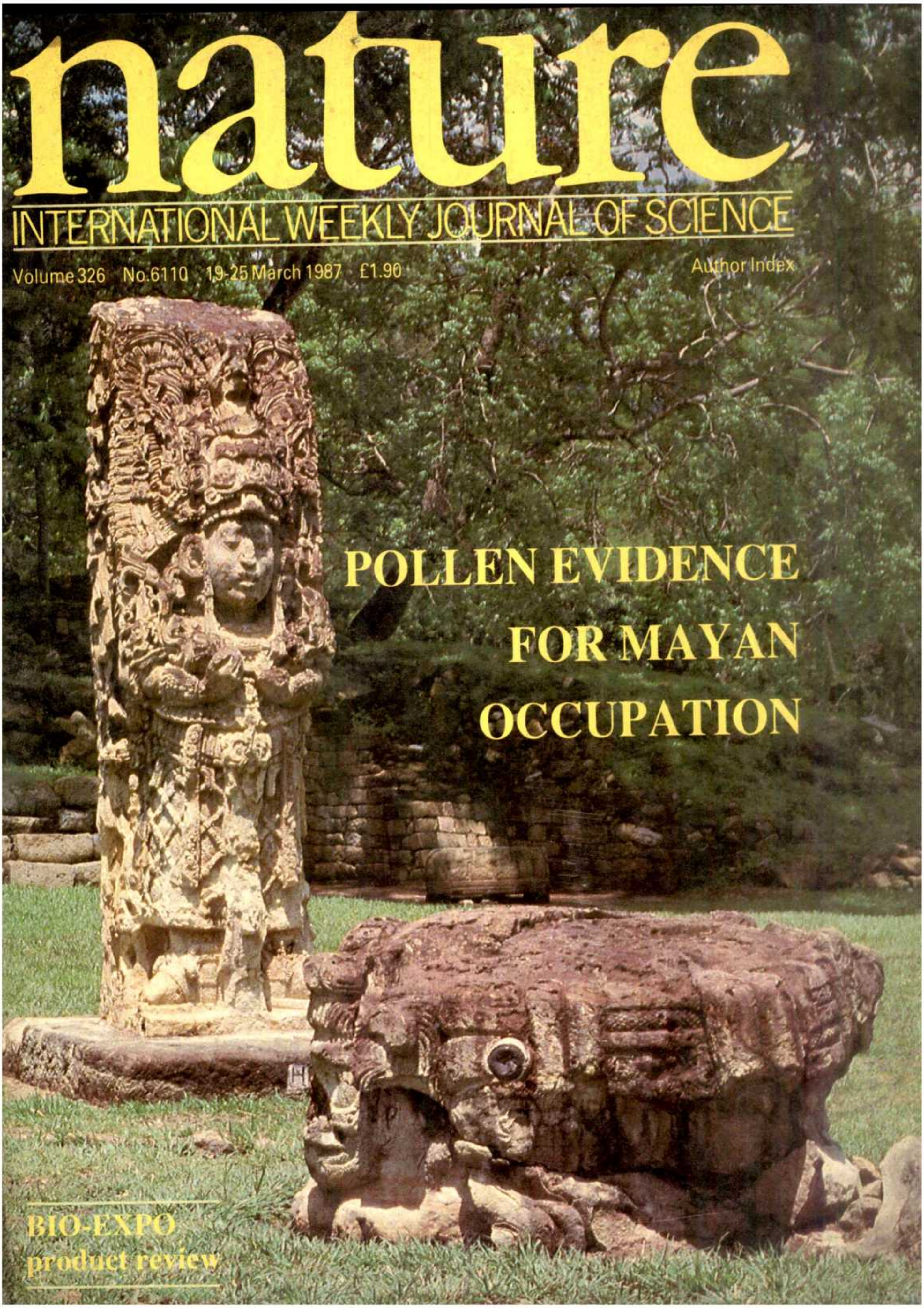

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BIO-EXPO product review

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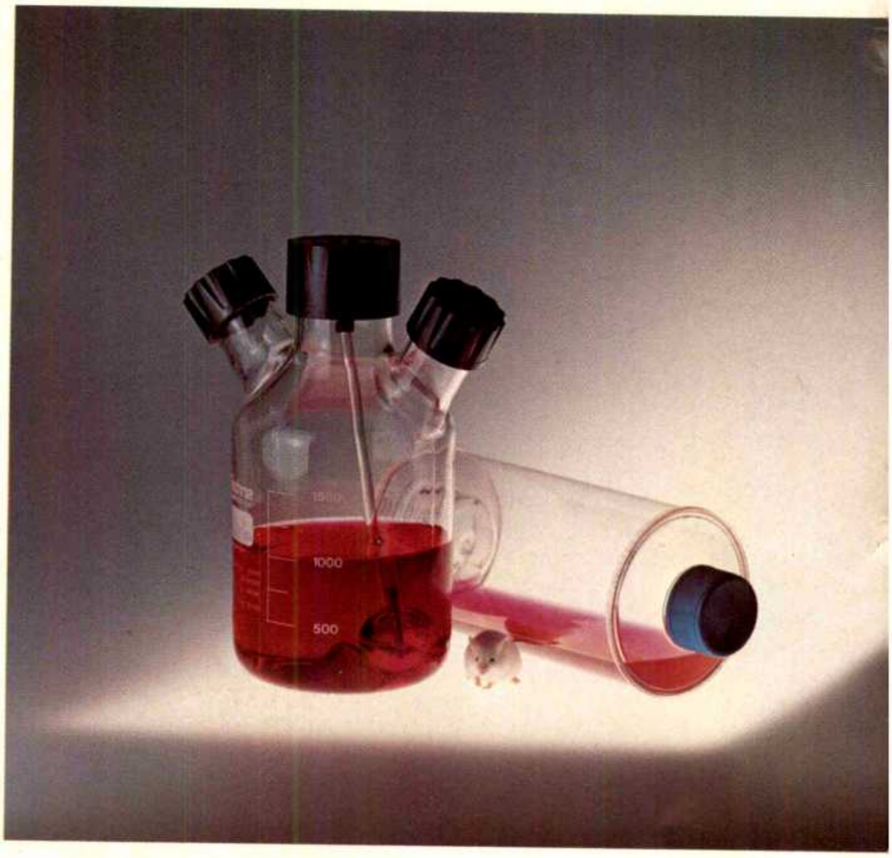
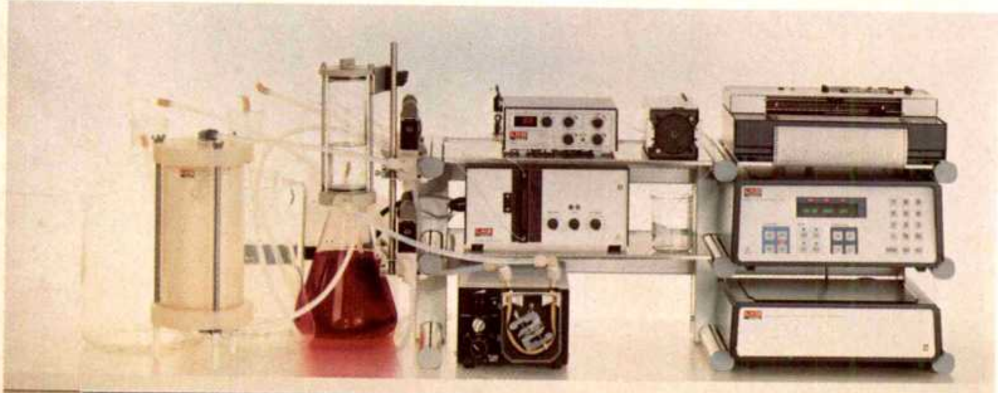
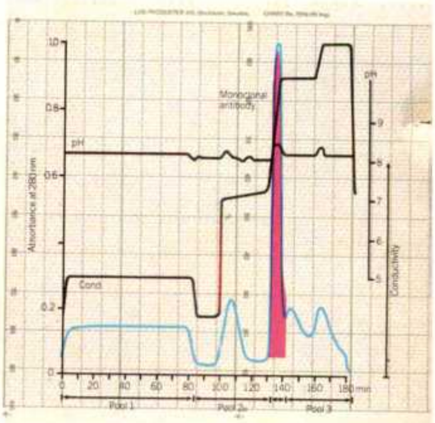
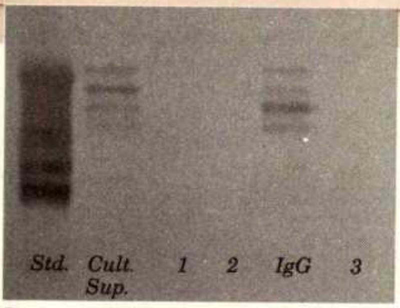




Pilot Chromatography System.



Scale-up purification of IgG antihuman urok nase using a QAE ZetaPrep 800 cartridg Yield: 1300 mg IgG. Flow rate: 160 ml/mi Sample volume: 10,000 ml cell culture supe natant.



Purity check by western blotting.

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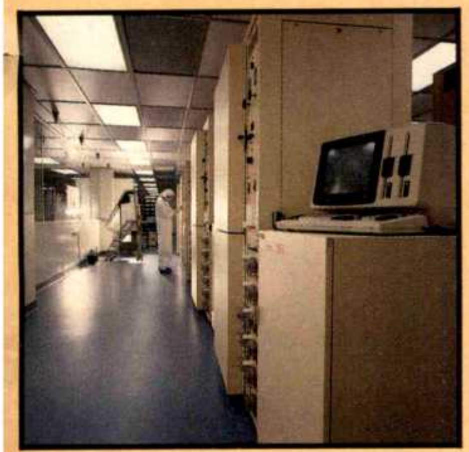

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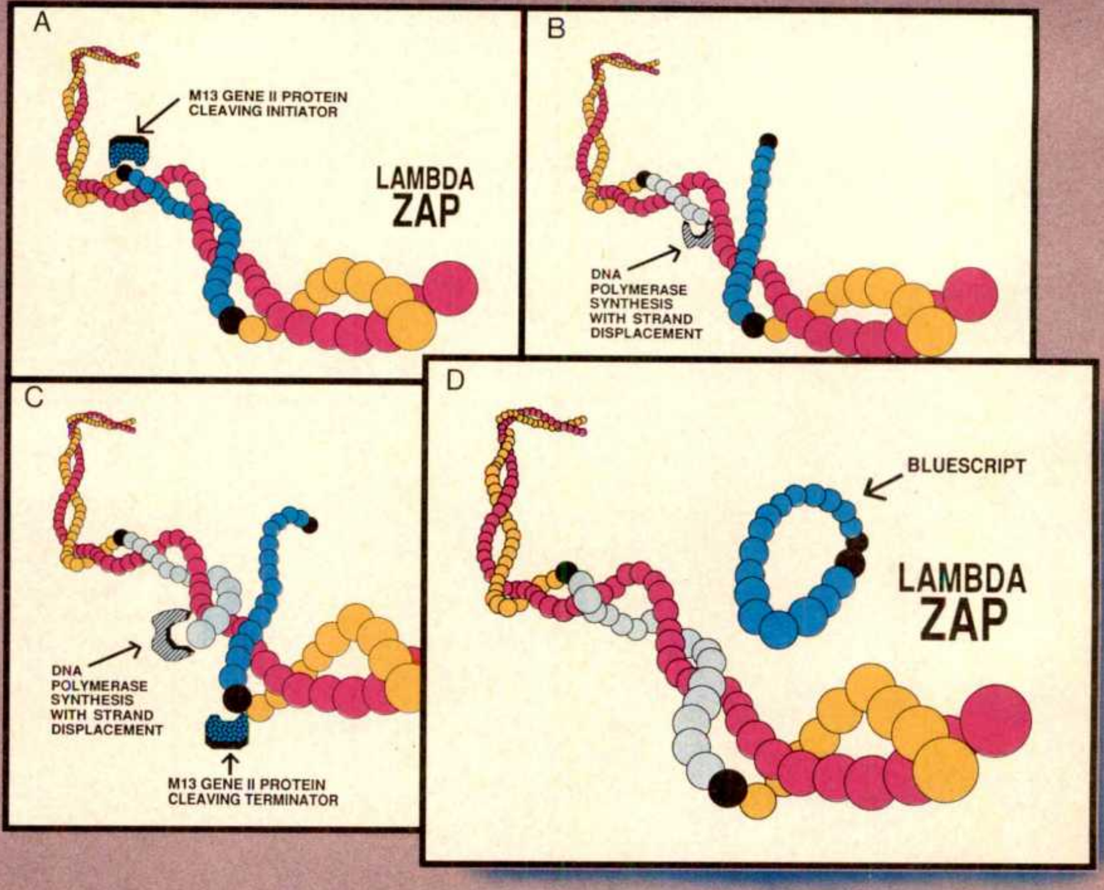
- No Lambda Phage Preps
- No Enzyme Digestions
- No Gel Electrophoresis
- No DNA Fragment Isolation
- No Ligations

Figure 1.

Automatic Excision of cDNA's from Lambda ZAP* to Bluescript


A
M13 GENE II PROTEIN CLEAVING INITIATOR
LAMBDA ZAP
B
DNA POLYMERASE SYNTHESIS WITH STRAND DISPLACEMENT
C
DNA POLYMERASE SYNTHESIS WITH STRAND DISPLACEMENT
M13 GENE II PROTEIN CLEAVING TERMINATOR
D
BLUESCRIPT
LAMBDA ZAP

A. M13 gene II protein recognizes the signal for the start of DNA synthesis and cleaves the DNA strand at the M13 recognition site.

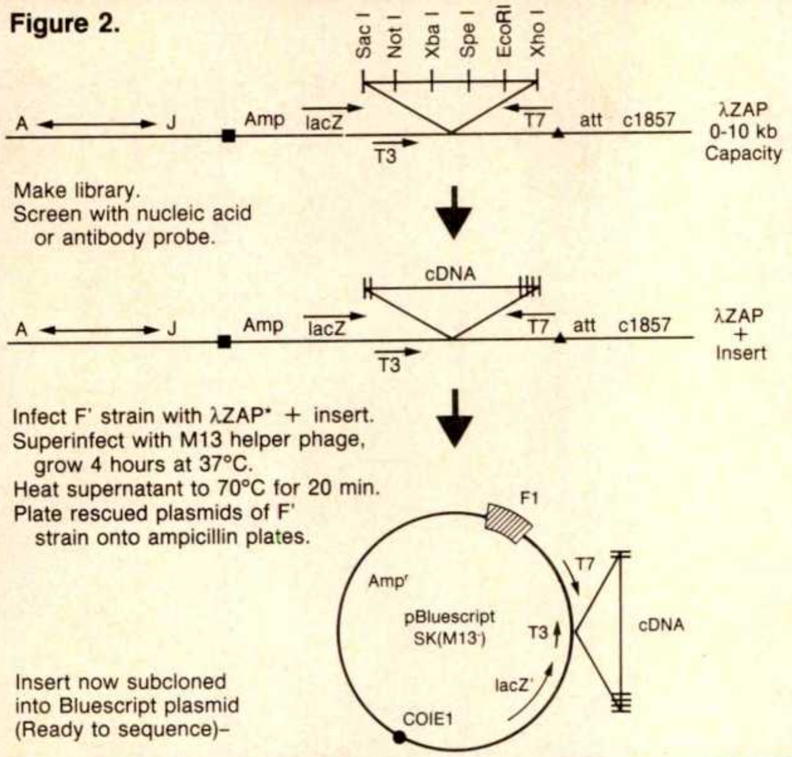
B. DNA polymerase synthesizes a new strand, displacing the existing strand.

C. M13 gene II protein recognizes the signal for termination of DNA synthesis and cleaves the DNA strand at the M13 recognition site.

D. M13 gene II protein converts the displaced strand of cDNA into a closed circular plasmid.

Breakthrough

Figure 2.



Make library.
Screen with nucleic acid
or antibody probe.

Infect F' strain with λZAP* + insert.
Superinfect with M13 helper phage,
grow 4 hours at 37°C.
Heat supernatant to 70°C for 20 min.
Plate rescued plasmids of F'
strain onto ampicillin plates.

Insert now subcloned
into Bluescript plasmid
(Ready to sequence)–

Outline of the Automatic Excision Process

The M13 helper phage supplies trans-acting proteins which recognize two separate signals positioned within the Lambda ZAP* arms. The two signals were derived from two overlapping halves of an f1 (M13) origin of replication. They are termed initiator (■), which denotes the signal for initiation of DNA synthesis, and terminator (▲) which denotes the signal for termination of DNA synthesis. Both of these signals are recognized by the M13 helper phage gene II protein. Following synthesis of a new DNA strand, the displaced strand is automatically circularized to form the Bluescript SK (M13⁻) plasmid containing the cloned insert. Since Bluescript SK (M13⁻) contains the signals for packaging as a M13 filamentous phage, the circularized plasmid is packaged and secreted from the cell. Bluescript plasmids are recovered by infecting an F' strain and plating on ampicillin plates, giving bacterial colonies.

KEY

A—J	: lambda structural genes
att	: lambda attachment site
■	: F1 initiator
▲	: F1 terminator
MCS	: Bluescript SK M13⁻ multiple cloning site, containing EcoRI, SacI, NotI, XboI, SpeI and XhoI unique sites in Lambda ZAP.
T3/T7	: RNA polymerase promoters

Stratagene's revolutionary new Lambda vector eliminates time consuming subcloning. cDNA's contained in Lambda ZAP* are automatically excised from the phage vector into the plasmid vector, Bluescript SK M13⁻ (see Figures 1 & 2) (1). The Bluescript SK M13⁻ vector (2) generated by this excision process has been specifically designed for experiments involving:

(1) Exonuclease III/mung bean nested deletions
(2) DNA and RNA sequencing
(3) Site-directed mutagenesis
(4) Synthesis of RNA probes
(5) Expression of proteins
(6) Isolation of single-stranded DNA
(7) Production of capped RNA transcripts
(8) Translation of capped transcripts.

In addition, Lambda ZAP* provides most of the advantages of other lambda expression vectors, such as Lambda gt11. The blue/white color selection on IPTG/X-GAL plates allows verification of the low background and high titers obtained from libraries made with Stratagene's pre-prepared Lambda ZAP* arms.

Lambda ZAP* provides six unique cloning sites which can hold inserts up to 10kb in length. Also, the XhoI, SpeI, and XbaI sites in Lambda ZAP* allow the cloning of MboI, BamHI, or HindIII digested DNA using the partial DNA fill-in technique of Stratagene's Lambda FIX.

(1) Short, J.M., Fernandez, J.M., Huse, W.D. & Sorge, J. (1987) Manuscript in preparation.
(2) Fernandez, J.M., Short, J.M., Renshaw, M., Huse, W.D. & Sorge, J. (1987) GENE (submitted).

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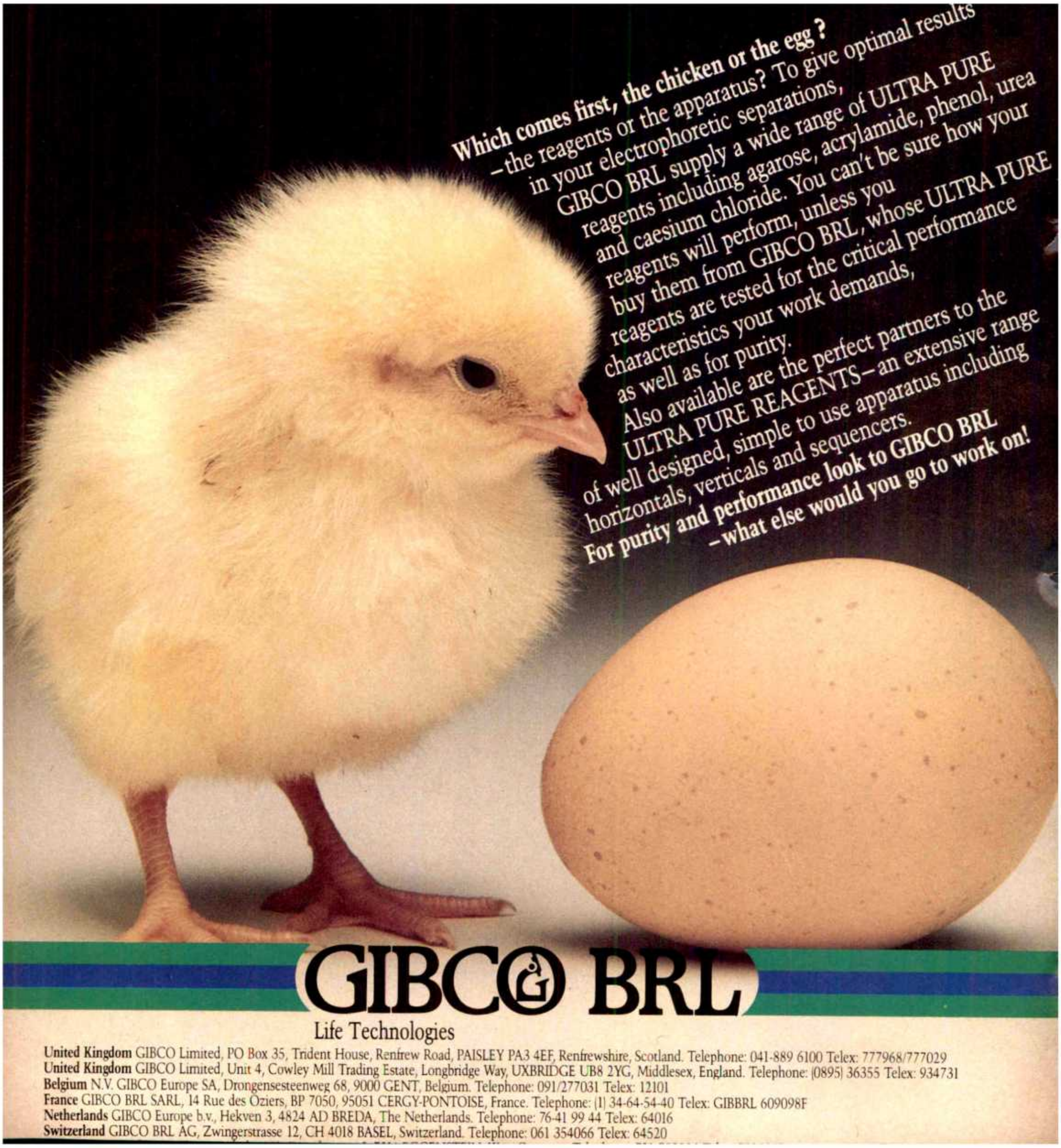

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nature
19 March 1987
Volume 326
Issue no. 6110

Stela and altar of 18 Rabbit, thirteenth king of Copan. Under 18 Rabbit and his successors, the Maya polity of Copan experienced unprecedented growth but the Copan political system collapsed abruptly at around AD 800. Now, pollen records provide evidence of agricultural activity as early as 4,500 yr BP, and as late as AD 1200 (see p.285 and News and Views).

THIS WEEK

■ Human trial for AIDS vaccine

. . . page 249

A medical researcher who injected himself with a possible AIDS vaccine has developed both humoral and cell-mediated immune responses.

■ Mouse model for Lesch–Nyhan disease

. . . pages 249, 292 and 295

Two methods of genetic manipulation of mouse embryos have produced young mice lacking the HPRT enzyme which is deficient in humans suffering from the disease.

■ Supernova news

. . . page 239

Physicists aid astronomers as neutrinos arrive from the supernova.

■ Space programme

. . . page 231

The European Space Agency announces a budget deficit for the year.

■ Female mate choice

. . . page 286

Female gray tree frogs can recognize not only the species but also the physical fitness of a male frog by the acoustic patterns of his call.

■ Lead pollution

. . . pages 244 and 278

The high levels of lead deposition in the most recent surface sediments of the mid-Atlantic Ocean floor arise from atmospheric industrial pollution.

■ Out of the ark

. . . page 245

Successful reintroduction of captive-bred animals into their indigenous habitats demands an appreciation of the genetic, ecological and behavioural problems that they will face.

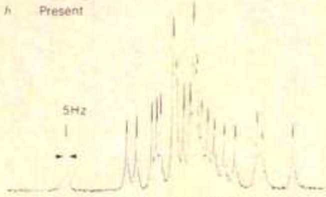
■ Stellar measurements

. . . page 257

Accurate measurement of stellar acoustic oscillation frequencies will allow the internal structures and ages of stars to be determined with much greater precision.

■ NMR prospects

. . . page 281



An order of magnitude improvement in resolution has been obtained for magic-angle spinning NMR spectra.

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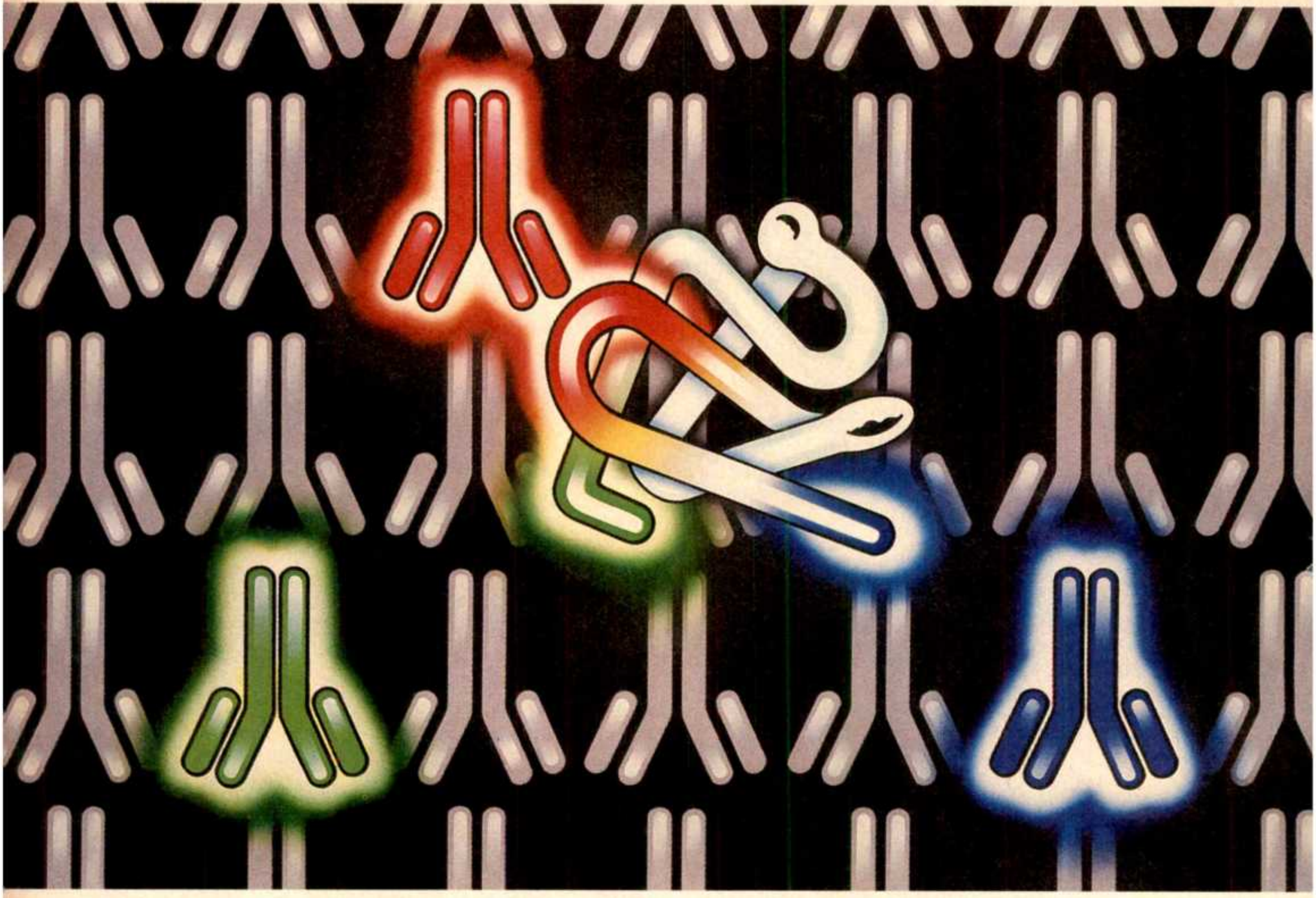

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THE TIME AND PLACE

Wednesday, May 6, at the Grand Hyatt Hotel in New York City (42nd Street and Lexington Avenue). The seminar begins at 9 a.m. with the closing reception at 5:30 p.m. For room reservations, call the hotel directly at 212/833-1234, ext. 1116, and mention The Bio/Technology Conference on Production Systems.

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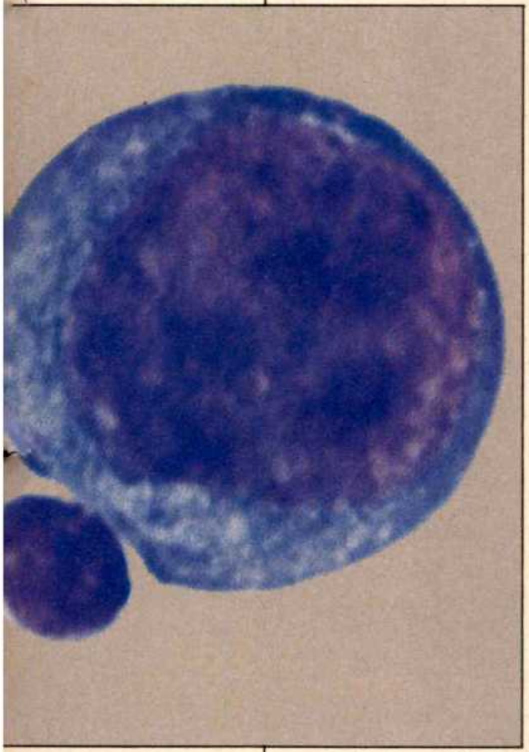

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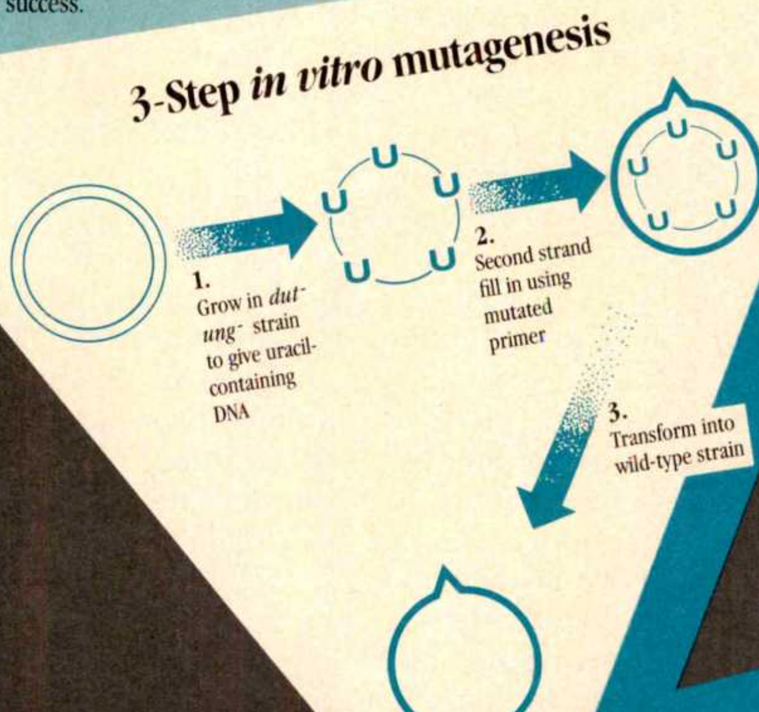
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1. Kunkel, T.A. *Proc. Natl. Acad. Sci. USA* **82**, 488-492. 1985.
2. Kunkel, T.A. Roberts, J.D. and Zakour, R.A. *Meth. Enzymol.*, in press. 1987.

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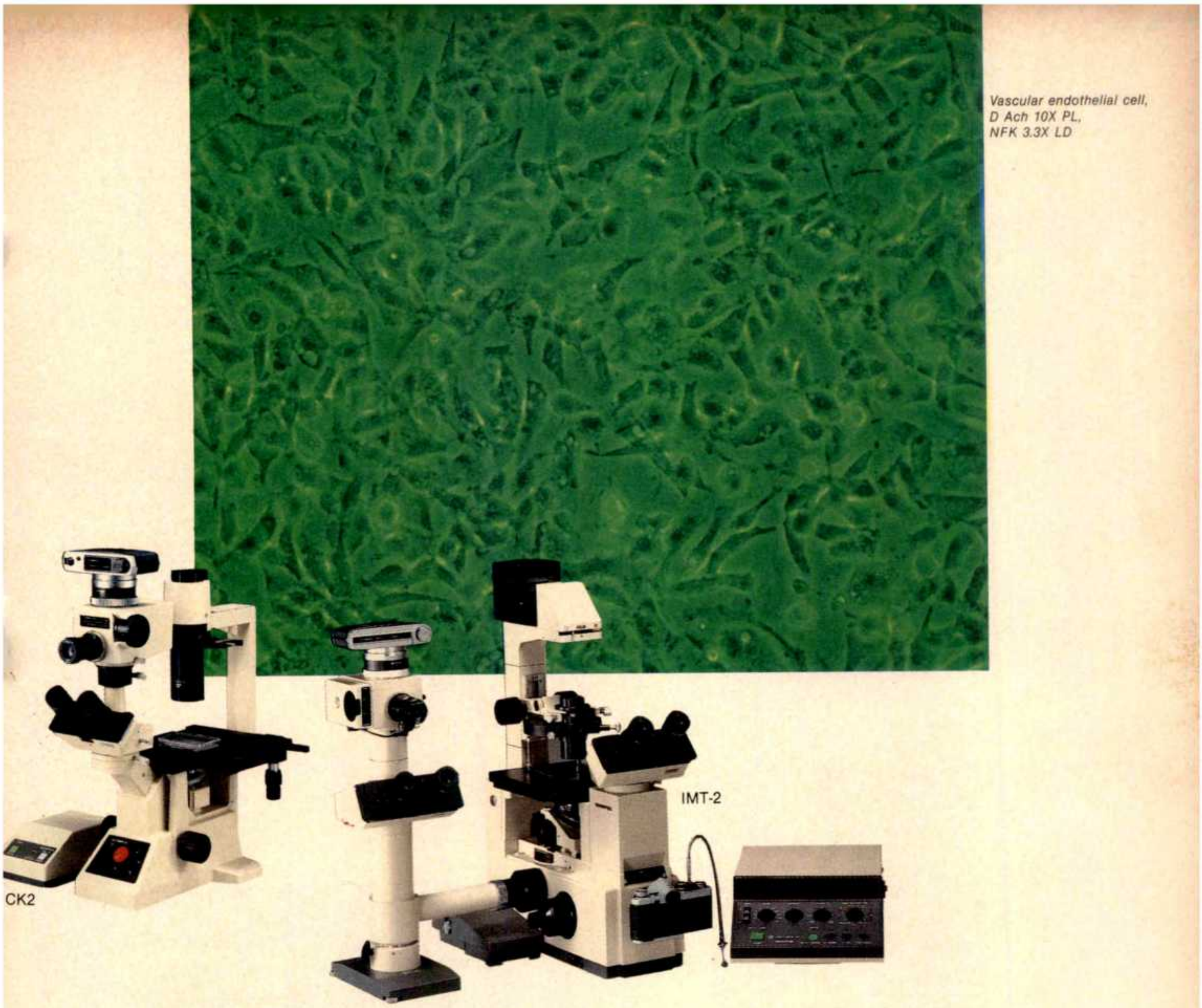

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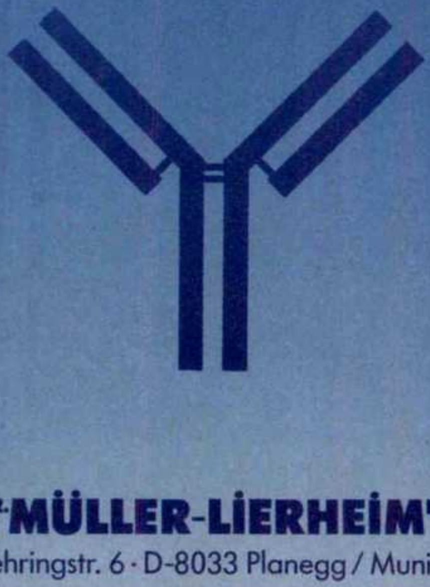

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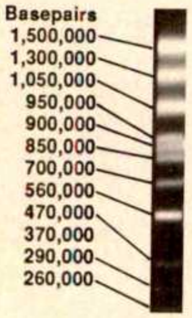

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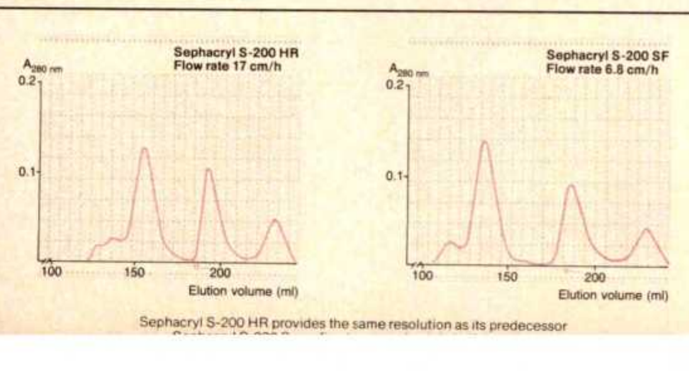

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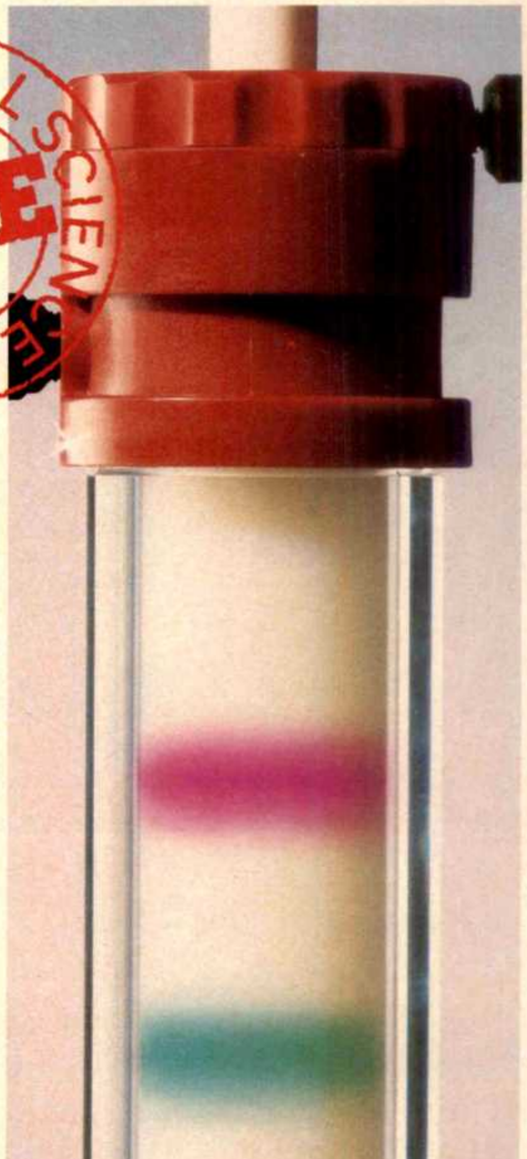

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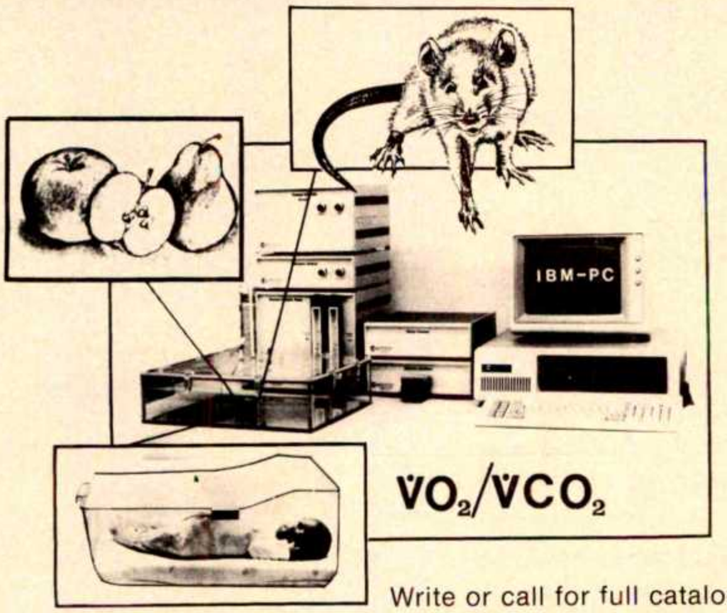

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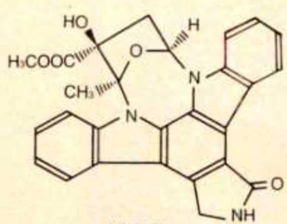

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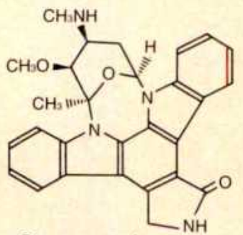

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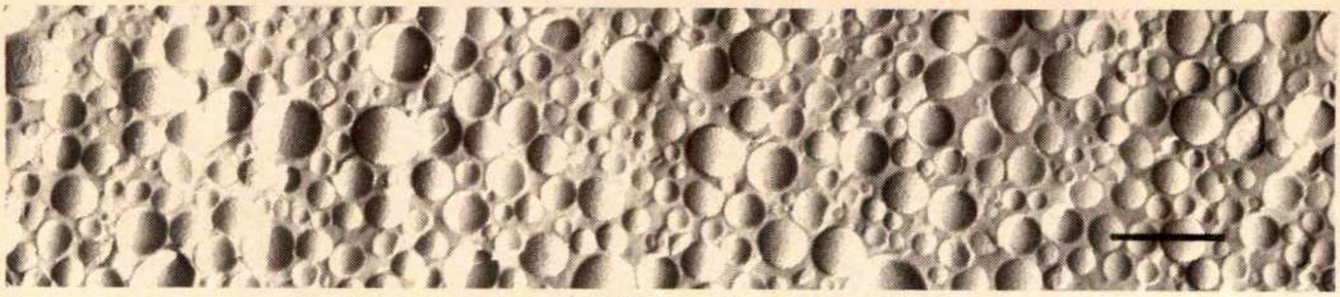

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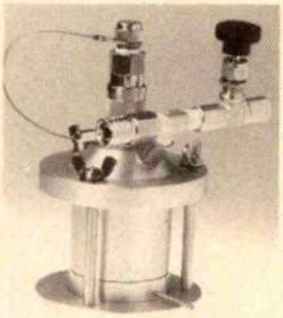

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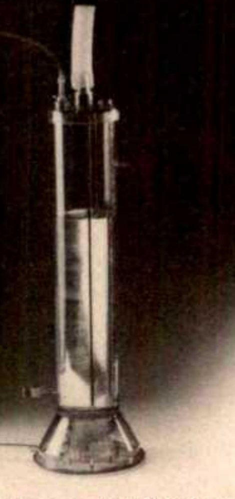

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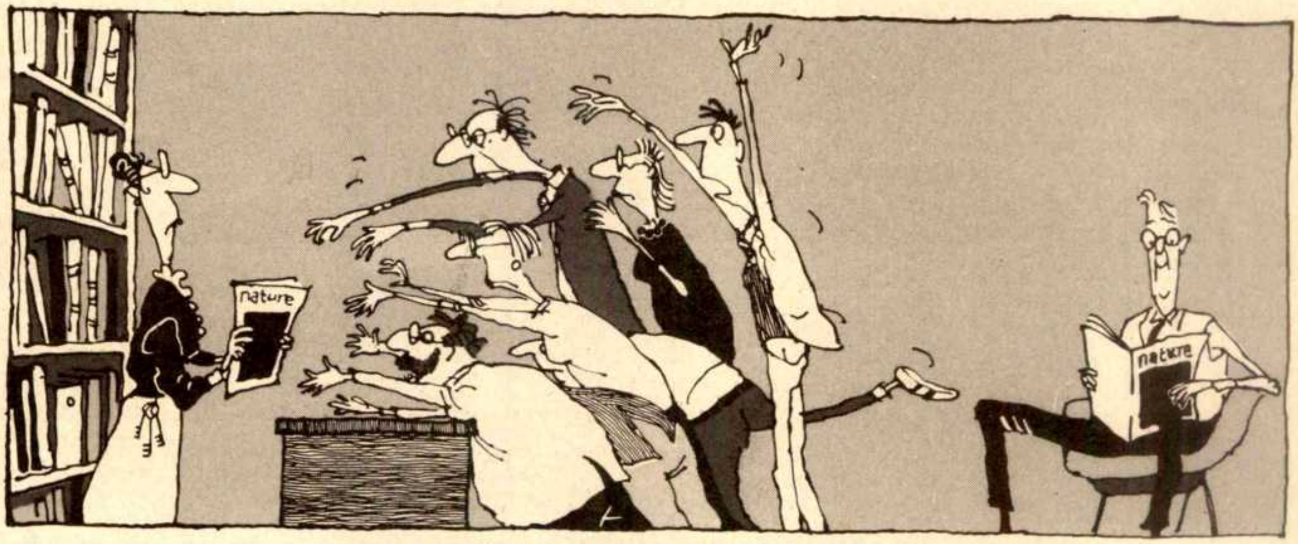

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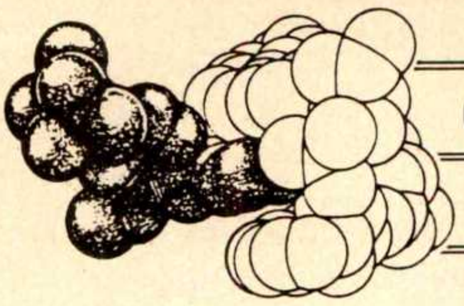

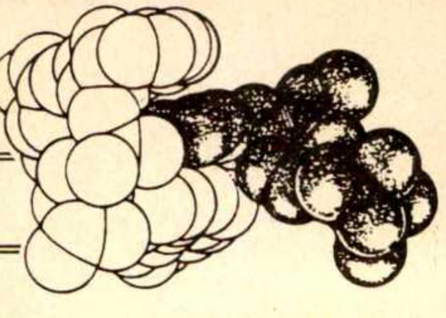

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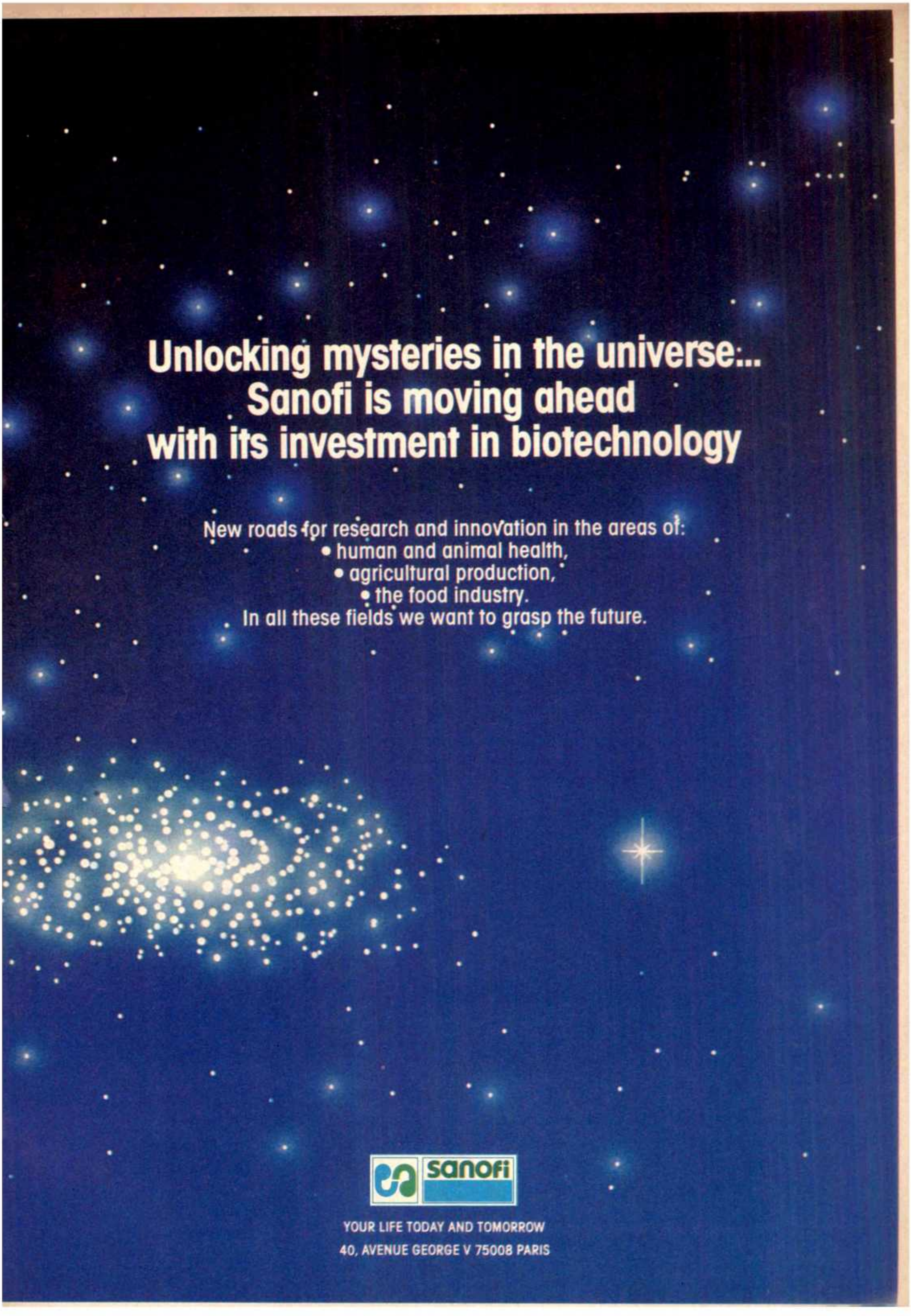

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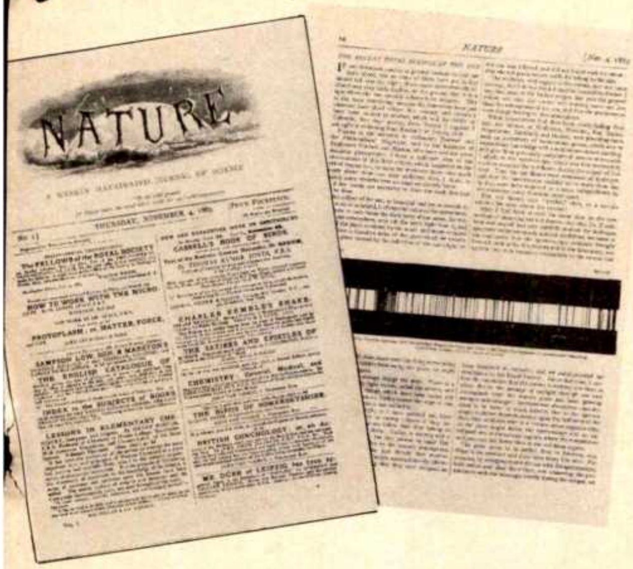
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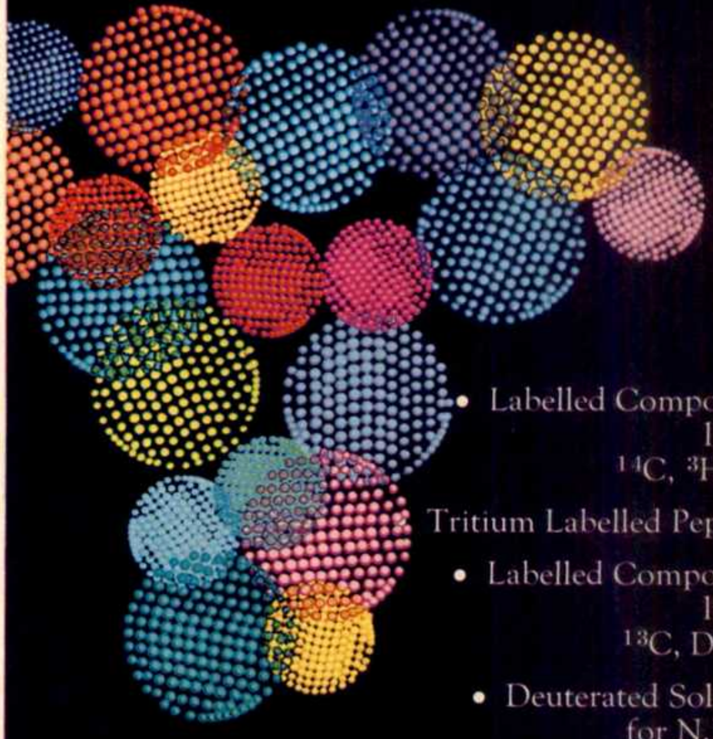

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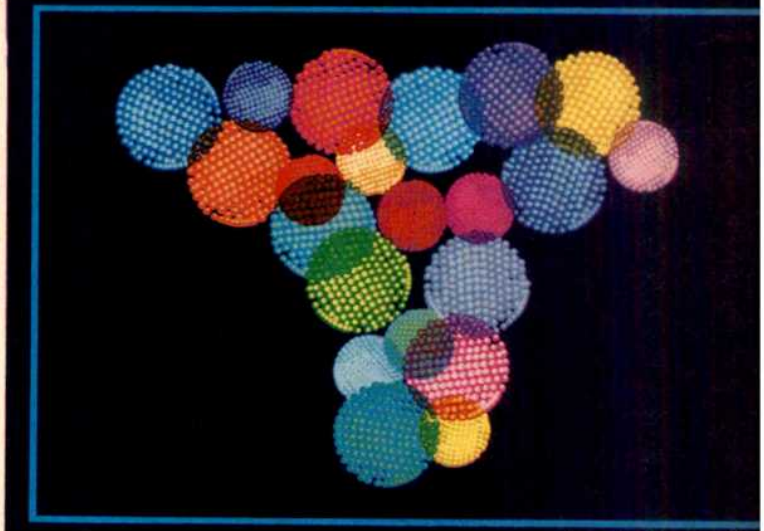

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The Vatican and embryology

Last week's Instruction to Roman Catholics on in vitro *fertilization and related matters is based on an unrealistic and insensitive appreciation of what modern science is like.*

THE Vatican, represented by the Congregation of the Faith, has done itself more harm than good with its statement last week on the lawful and illicit uses of new techniques in human embryology. While claiming a foundation in modern science, the statement emerges as a restrictive prescription out of tune with the temper of science as it is. But natural scoffers should pause, at least for long enough to read the document put out from Rome, before denouncing it as irrelevant. At the least, the document could further confuse several important issues of public policy. If taken at its face value it could be a drag on research and the therapeutic techniques that will follow.

Among the several recommendations in what is technically an Instruction to members of the Church, but which is also intended as a legislative programme for sympathetic civil governments, the most striking is the interdiction of the use of two spouses' own gametes as the starting-point for *in vitro* fertilization (IVF). Everybody knows that Rome is against contraception and abortion, but why, people are asking, should two mutually faithful people who happen to be mutually infertile be deprived of children when there is a technique that may give them relief from their frustration? As may be expected, the Vatican's argument is subtle, consistent and consonant with previous declarations on related matters. Its defect is an over-simple view of science, which is nevertheless offered as the foundation of last week's Instruction.

The new Instruction is derived, with only a little contemporary tuning, from two moral principles — that the lives of individual human beings should be respected absolutely and that there must be an unbreakable and reciprocal connection between all sexual acts and the process of procreation. The first principle is nowhere disputed, although the Vatican characteristically goes further than would most (but not all) biologists in its insistence that life begins with the union of two haploid gametes to form a diploid zygote. The second principle, on the other hand, is distinctively Roman Catholic in its strict application, and is the origin of the Church's condemnation of masturbation and contraception, both practices lacking what the Instruction describes as "openness to procreation".

Science affirmed

For the past several years, one of the dilemmas forced on the Vatican by developments in biology has been the question whether the second principle applies in reverse, whether procreation without natural conjugal intercourse should be discouraged or even disallowed. The decision, that it must be forbidden, while amply supported in the Instruction with references to recent and past doctrinal authorities is where the trouble starts.

Some time ago, the Vatican demanded to know of Galileo whether it was his opinion that the Sun goes round the Earth or the other way about. The legend is that the old man chose to tell what he believed to be a lie even though, in these relativistic days, he would have been well within his rights to answer "either or neither, as you wish". The Church has learned the lesson of that sad encounter and is now ready to embrace scientific discovery even if, on the evidence of the Instruction, selectively and even perversely. Unfortunately, the conclusion that the two-way application of the doctrine on conjugal intercourse and procreation is, even in the Church's own terms, both unnecessary and a hostage to the fortune of future scientific discovery. In reaching its conclusion, the Vatican has relied on too literal an appreciation of what science is about. It sets too much store by particular discoveries and too little by the objective of the enterprise, which is understanding.

This is nicely illustrated by the issue of deciding when, in the course of human development, life begins. Last week's Instruction takes as its starting-point "recent findings of human biological science" that all zygotes are genetically distinctive, so that the "biological identity of a new human individual is already constituted" once fertilization is complete. Mindful, no doubt, of the old arguments about when the soul may be considered to enter the body, the Instruction admits that there is "no experimental datum" to help decide, but asks the rhetorical question "how could a human individual not be a human person?".

Life's beginning

The case for banning even "homologous" IVF, where the parents provide the gametes, is built on this foundation. The first principle of the absolute sanctity of life after fertilization provides an elementary justification; IVF at present entails the production of 'spare' embryos that cannot be discarded without breaking the Church's law. The Instruction, anticipating that this objection may in future be met by the improvement of clinical technique, goes on to say that IVF would even then remain illicit because of the principle of the indissoluble linkage of conjugal intercourse and procreation. First, it says, because IVF entrusts "the life and identity" of the embryo into the "power of physicians and biologists", establishing the "domination of technology over the origin and the destiny of the human person", it offends intolerably against that person's dignity. Second, and in any case, because IVF is by definition different from conjugal intercourse, children emerging from the process are deprived of "proper perfection". Tolerantly, however, the Instruction reminds members of the Church that even children unfortunate enough to be conceived in this way "must be brought up with love" and couples who happen to be infertile that marriage does not bring "the right to have a child" but only the right "to perform those natural acts which are intrinsically directed towards procreation".

These conclusions will mystify most who are not Roman Catholics (and disappoint many who are). The remark that each embryo's DNA is distinctive is indisputable, but does not lead logically to the conclusion that each embryo is a "new human individual"; for the time being, it seems that the hospitality of a uterus, most probably a human uterus, is required to accomplish that. (The Instruction also bans trans-specific gestation and the artificial uterus.) If further investigation should show, which is not improbable, that the sojourn of an embryo in a uterus is somehow indispensable to its maturation, would the whole set of interdictions promulgated last week then collapse like a house of cards? Of course not, but for reasons quite different from what the Instruction calls the "cold logic" of its prescriptions.

Ironically, while the Congregation of the Faith has abandoned the old quest for a knowledge of when the soul enters the body, others have been moving in the opposite direction, but from different motives. In laboratories, for example, it is important to know when the development of artificially fertilized embryos must be stopped for fear of accumulating unwanted and inviable human individuals in Petri dishes. For want of a sufficient knowledge of embryogenesis, responsible civil jurisdictions have settled for time-limits acknowledged to be somewhat arbitrary, such as the 14-day limit proposed three years ago in Britain by the Warnock committee. Last week's Instruction, which also forbids research with, as distinct from therapy on, living embryos would enormously impede the search for a more viable understanding of where the limits should be drawn so as not to offend those who work in laboratories, and who are no readier than the next person to throw living "human individuals" away simply because they can be seen only with a microscope. In its preamble, the document asserts that science cannot be held to be "morally neutral", and insists that "science without conscience" is offensive. Would it not have been charitable to acknowledge that researchers have been among the most active in the search for a seemly definition of when life begins?

That would also have been prudent. Over the years, the Church has accumulated a great burden for itself and its members by its position on the absolute sanctity of human life. Morally and even intellectually, the position deserves respect. But what if it is unnecessary? What if it is equally respectable, morally and intellectually, to found a view of when life begins on an account of early embryogenesis more rounded than that now accessible based on an appreciation of the development of the early nervous system in the three weeks after fertilization, on the emergence of distinctively mammalian characteristics in the days that follow or on other observations yet to be carried out? In these post-galilean times, the only certainty is that there will be no simple way of drawing a distinction between the living and the human, but outsiders cannot but ask whether the Church itself would not benefit from a successful search towards that end. Then it need no longer be impaled on the hooks of its policy on contraception and, now, on IVF.

Cruel doctrine

Much the same is true of what the Instruction says of the link between conjugal intercourse and procreation. There is a body of opinion much wider than the Church that the stability of the family should be an objective of public policy. In many jurisdictions, the principle is accepted that there is no inalienable right of couples "to have" children. But can it seriously be held, in the face of evidence from social scientists and clinical psychiatrists, that the moral error in the relief of the frustrations of sterile marriages outweighs the evident benefits of physicians' admittedly artificial intervention? This is a cruel doctrine, as the Instruction admits. Is it really necessary, and can it be wise?

The Vatican, of course, will say that prudence should be no part of its calculation, only moral rectitude. By the Church's own lights, nobody can quarrel with that position. The issue is simply whether the experimental basis of its moral position is as secure as the Congregation of the Faith asserts. Certainly it is curious that a document purporting to reflect modern scientific understanding, and with its interdiction of "science without conscience", should repeat the Church's traditional view of the sharp distinction between the human and other species, thereby apparently providing a licence for animal experiments much broader than seemly societies would now allow. Many loyal members of the Church will now be asking whether the Vatican is leading them up a blind alley from which only humiliating retreat is possible. For outsiders, the important questions are whether the interdictions of the Instruction will impede the fuller understanding of human embryology and whether the Instruction's oblique incitement to civil disobedience in the pursuit of strict legislation carries weight. □

European research crisis

The European Communities' research programme will be decided next week too lightheartedly.

THERE is something in the view that Europe has been living in a crisis of transition since the time of Charlemagne. Europe's problem is that of reconciling its sense that united action will be beneficial with the wayward determination of its several national states that they should individually say what form united action should take. The European Communities have been slaves to this dilemma since their creation 30 years ago. Now the problem afflicts their programme for research. A plan to spend more than 8,000 million European currency units (roughly the same number of US dollars) over the five years beginning in January was put forward last year by the European Commission's chairman, M. Jacques Delors, as a test of Europe's willingness to take the Commission seriously. In the event, the British cancelled the meeting last December at which the issue should have been decided. Next week, in Brussels, the member governments will probably settle for a more modest programme, lopping 1,000 million ECU off the original bill.

Will that mean that the cause of common action has been advanced? Or set back on its heels? All interested parties will no doubt form their own opinions. The sad truth, however, is that these will be out of tune with the opinions of most working researchers in European states that next week's compromise will not presage the evolution of the common research programme that Europe needs. The objection to the Commission's plan, even as modified for next week's decision, is that only some of its components carry the promise of succeeding as the Commission would wish.

Some features of the proposals are splendid. Only the Commission, for example, could have given Europe a chance of contributing significantly to the development of fusion reactors by means of its sponsorship of the Joint European Torus (JET); it is to be hoped that the outcome of that experiment will indeed allow work to begin of the detailed design of NET (Next European Torus) in 1990 as the Commission hopes. The case for this project, which will consume a seventh of the research budget, is that no single member of the Communities would have chosen to mount it from its own resources. But member states have been ready to cede precedence to the Commission because the goal is so distant that national competitive instincts are not directly aroused. But it will not be long before the Commission finds that it has earned a substantial fund of goodwill, and perhaps even a willingness by member states to cede it further responsibilities, for having carried through this project.

The same, unfortunately, cannot be said of other components of the research programme. That for stimulating pre-commercial development in computer technology (called ESPRIT) is big enough, at 2,050 million ECU but not decisively so. Indeed, this spending is only a small part of what European governments and companies are likely to spend in this field over the next five years. Yet it remains to be seen whether the Commission's programme will add something distinctive to the pattern of work elsewhere. More often, the programme appears to those who work with it as a means of augmenting their own sources of finance by means of applications to Brussels. In an ideal world, the Commission would have been required to produce a much more formal and rigorous evaluation of the work already carried out before being given a second tranche of funds.

Much the same applies to its other proposals for the next five years. It is hard to believe that much will come of the Commission's proposals in biotechnology or in the battle against AIDS (although there is a data-collection job to be done by somebody); national governments are too much engaged already. It may for the long run be more significant that the Commission now offers a programme for making the research institutions of its members more flexible, and their people more mobile. □

Europe underestimates cost of space programme

- ESA Paris meeting to review budget
- Trading national space projects

London

THE European Space Agency (ESA) has underestimated by more than £600 million (1,100 million European Currency Units, ECU) the amount needed annually to fund its new space programme, precipitating a major rescheduling of its proposed international research projects.

The British National Space Centre (BNSC) says the cost of the programme will be 2,800 million ECU, a third more than the previously expected cost.

The 13 nations that comprise ESA will need to curtail their space programme substantially, or demand even more money from the members. An ESA council meeting is scheduled for the end of this week in Paris, when members will be asked to declare their commitment to the space plan and to discuss a revamped programme.

ESA had agreed in Rome in 1985 to increase the European space budget by nearly 70 per cent over three years. The basic outline of the programme had also been agreed, at an estimated cost of 1,750 million ECU a year at the peak of the programme in 1990.

There is likely to be considerable friction over the projects to be jettisoned to bring down the cost of the programme. France is keen to develop the Hermes spaceplane and the Ariane heavy launcher; West Germany, Italy and the United Kingdom have given support to the space station project.

BNSC still awaits a response to the 15-year space plan presented to the government last summer (see *Nature* **324**, 197; 1986). Attempts were made two weeks ago to get together all the relevant government departments to agree a space budget — expected to be about £200 million a year, with half spent within the United Kingdom — but it failed to take place. Ministerial discussions are still in progress.

The UK space programme is at present largely funded through the Departments of Trade and Industry and of the Environment and the Ministry of Defence. The BNSC plan would bring all this support under its own control, an option likely to be vigorously opposed by the relevant ministers.

The ESA meeting this week will demand not only that the members make a commitment to Europe's long-term space hopes but will also be one of many to prepare for an ESA ministerial summit scheduled for June.

ESA members have been particularly concerned about developing space for peaceful use, and are still disturbed about US intentions to devote military resources to the space station, previously considered a civil project (see *Nature* **325**, 563; 1987).

Norway and Austria became full members of ESA this year, joining West Germany, Ireland, France, Denmark, the United Kingdom, the Netherlands, Spain, Italy, Sweden, Switzerland and Belgium. Finland has associate member status and Canada has a 'special arrangement'.

The shortfall in the ESA budget (costs at 1985 prices) could provoke serious disharmony within the group. Members are keen to pursue space technology, but each project is assessed individually; horse trading will begin this week, with each country trying to ensure its share of the research and development cake.

The ESA members are well aware of the opportunity now afforded them as a result of the mishaps experienced by the US space shuttle programme. The development of the Ariane V heavy launcher, in particular, could offer substantial commercial opportunities to the Europeans for launching civil communications satellites, although fierce competition is expected from Japan, China and the Soviet Union.

Europe has also been keen to promote more use of the European Communications Satellite network. Two satellites have already been launched and carry television and telecommunications signals to most parts of Western Europe.

The race within Europe to gain a foothold in direct broadcasting by satellite is now under way, with France, West Germany and the United Kingdom in the front line. Reliable launchers and space communication technology will be fundamental in ensuring the success of these programmes. **Bill Johnstone**

British Labour Party science policy

ON Thursday 26 March 1987, *Nature* will be providing a forum in London at which Labour Party representatives will outline their policy on science.

As space is limited, anybody wishing to attend should telephone Neil Crombie in London on 01-836 6633, extension 2480. ☐

Protropin status questioned by FDA decision

Washington

A DECISION by the US Food and Drug Administration (FDA) may cause the biotechnology company Genentech to lose its position as market leader in sales of recombinant human growth hormone (rhGH). Last week, FDA granted approval to Eli Lilly & Co. to market another version of the hormone in a move that could change the interpretation of the 1983 Orphan Drug Act.

Genentech's rhGH, tradenamed Protropin, is a 192-amino-acid protein, consisting of the 191-amino-acid hormone normally found in humans plus an extra methionine residue at the amino-terminal end, an artefact of recombinant production in *Escherichia coli*.

Protropin was granted approval by FDA in 1985 under the Orphan Drug Act, as a treatment for children with insufficient growth hormone. The act provides financial incentive for manufacturers to develop drugs for rare diseases, where they cannot expect to recoup development costs, let alone make a profit and entitles the manufacturer to exclusive marketing rights for seven years.

But human growth hormone sales could reach $70–80 million in the next year, and many believe orphan drug status should not have been granted. Last year, all of Genentech's $43.6 million product income resulted from sales of Protropin. Such success prompted three other companies, Ares-Serono, Bio-Technology General, and Eli Lilly, to apply for marketing approval of variants of rhGH lacking the initiation methionine.

Eli Lilly's drug, Humatrope, is also produced in *E. coli*, but does not bear the amino-terminal methionine. Eli Lilly claims its methionyl-free rhGH is less antigenic than Genentech's, and because it has a different structure, it should also be given orphan drug status. Last week the FDA concurred, and gave Humatrope marketing approval.

Genentech is contesting that approval infringes the seven-year marketing exclusivity it is entitled to for Protropin. On 6 March, Genentech filed suit against FDA in federal court in Washington, to force FDA to clarify how the act applies to genetically-engineered drugs. It also filed a preliminary injunction against Eli Lilly to prevent them from marketing Humatrope, and this hearing is scheduled for 26 March. Genentech is also seeking orphan drug approval for its own methionyl-free version of rhGH. The result of all of this could be a complete redefinition of the Orphan Drug Act. **Carol Ezzell**

AIDS becomes a notifiable disease in Japan despite protests

Tokyo

JAPAN is about to introduce legislation to make AIDS (acquired immune deficiency syndrome) a notifiable disease. But critics claim that the law will be a grave infringement of human rights.

The following are the main provisions of the AIDS bill: doctors must report the age, sex and source of infection of AIDS-virus carriers to the prefectural government concerned within 7 days; if a doctor judges that a patient may ignore his instructions, the doctor should immediately report the name and address of the patient to prefectural authorities; the prefectural governors are authorized to recommend or order AIDS tests and medical checkups for people infected or suspected of being infected with AIDS, and a maximum fine of Y100,000 will be imposed on those who refuse; doctors and public servants who fail to keep the secrets of patients will be subject to maximum imprisonment of up to one year or a fine of less than Y300,000; foreigners infected with AIDS will not be allowed into Japan, for the time being.

Health and Welfare Ministry officials responsible for drawing up the law have found themselves pulled in two directions. On the one hand, some of their scientific advisers, who are involved in the treatment of haemophiliac AIDS patients, wish to guard the privacy of patients; on the other, government officials and politicians are pressing for draconian measures to halt the spread of the disease.

Until very recently, most Japanese regarded AIDS as a disease confined to foreigners and a few Japanese homosexuals and haemophiliacs. But the death of a female prostitute in Kobe in January and revelations in the press about a female AIDS-carrier in Kochi have sent government officials rushing to draft anti-AIDS legislation.

According to press reports, the woman in Kochi ignored her doctor's advice, became pregnant without informing her husband that she was an AIDS carrier, and insisted on giving birth even though there is a high risk the child has been infected. (The birth was announced on Monday, but blood tests of the infant will take several weeks.)

In an early draft of the law, those infected with AIDS who engage in 'dangerous acts' (giving blood or having sexual relations) in the full knowledge that they are infected would have been subject to a maximum fine of Y300,000 ($2,000) or imprisonment for up to one year. And doctors who failed to report AIDS carriers to prefectural authorities would be subject to similar punishment. But protests that these provisions constitute an infringement of human rights led the ministry to drop these clauses from the final draft.

Hoei Ohama, chairman of the recently formed subcommittee on AIDS, of the ruling Liberal Democratic Party (LDP), told reporters that "it is more important to prevent the spread of AIDS than to protect the privacy of high-risk groups" and went on to add that "if we respect the human rights of one person, we are depriving 99 others of the right to live".

Some Justice Ministry officials have even suggested that people infected with AIDS who knowingly pass on the virus to others should face injury or murder charges under existing criminal law.

The Health and Welfare Ministry says the new law will protect the privacy of AIDS sufferers as long as they follow doctors' advice. But what is not made clear is how those secondarily infected with AIDS will be tracked down while preserving that privacy. The clause banning the entry of foreigners is also extremely vaguely worded and there is no description of how it will be enforced

The National Association of Haemophiliacs has condemned the legislation as "inhumane". But if some members of the LDP have their way the law will be strengthened during Diet debate. Tatsuo Ozawa, leader of an AIDS study delegation to the United States, announced in Washington on Friday that he will recommend compulsory medical checks for those employed in massage parlours, and strengthening of the enforcement of AIDS preventative measures while developing measures to protect human rights.

David Swinbanks

AIDS education programme in Japan

Tokyo

JAPAN is taking several steps to combat AIDS (acquired immune deficiency syndrome). The Health and Welfare Ministry has distributed a 100-page booklet on the disease to prefectural governments across the country; the Education Ministry has instructed education boards to initiate AIDS education from the primary school level up; and television coverage on the disease is now extensive. AIDS-related stocks have soared on the Tokyo stock exchange.

Now Tatsuo Ozawa, a former Health and Welfare Minister who led a mission to the United States last week to study AIDS, has proposed the formation of a private foundation to produce education films and video tapes and to maintain a free phone-counselling service. David Swinbanks

Tests for AIDS in pregnancy on offer to all

Munich

VOLUNTARY testing for AIDS (acquired immune deficiency syndrome) should be available for all pregnant women. That was the conclusion of an international symposium on infectious diseases in gynaecology and obstetrics held at the University of Munich earlier in the month. The bread-and-butter of the conference, which brought together more than 500 gynaecologists from nine countries, were topics such as toxic-shock syndrome and premature delivery.

A pilot programme of AIDS testing among expectant mothers has already begun in the university hospitals of Munich. According to Ernst Weissenbacher, the organizer of the conference and professor of medicine at the university, "almost nobody" has refused to be tested.

Of the estimated 100,000 carriers of the AIDS virus (HIV) in West Germany, between one and seven per cent are thought to be women. Virtually all carriers of the infection are members of high-risk groups. At present, the results of AIDS tests are confidential between patients and their physicians which, according to Weissenbacher, helps to explain why pregnant women are ready to volunteer for tests. But Bavarian physicians may soon be required to report carriers of the AIDS infection to local health authorities in which case, says Weissenbacher, the situation could change.

The government of Bavaria has put forward one of the most far-reaching programmes of AIDS testing yet proposed. Under intended legislation, tests would be mandatory for applicants for public service jobs as well as those "in danger of infecting others", including members of high-risk groups such as prostitutes and intravenous drug users and their associates.

Under the planned law, those found to be carriers of the infection would be required to inform their physicians and their sexual partners of their condition. The Bavarian government's plans are vigourously opposed by the Minister of Health in the federal government, Rita Süssmuth.

Steven Dickman

New director at BGS

London

THE UK Natural Environment Research Council (NERC) has appointed Mr F.G. Larminie to a two-year term as director of the British Geological Survey from September, on the retirement of the present director Mr G.I. Lumsden. □

Cattle farming in the desert heat an unexpected success for Arabs

Riyadh

HOLSTEIN cattle, originally bred to suit the temperate climate and green pastures of the Netherlands, would not seem to be the ideal choice for a dairy herd in the scorching Saudi-Arabian desert. But 17,600 Holsteins on a farm 50 miles south of Riyadh, the country's capital, not only survive regular summer temperatures of 45–50° C, but are good milk-producers.

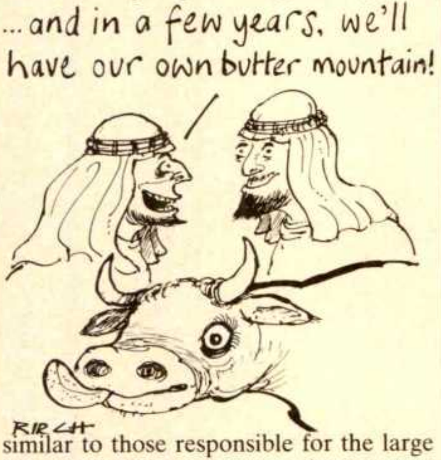
Even more remarkably, what is claimed to be the largest herd of cattle in the world is surrounded by 'greened' desert that produces about two-thirds of the cattle feed. And tucked in among the cattle pens is a processing plant that processes the daily 125,000 litres of milk produced by the herd, turning much of it into laban, the 'liquid yogurt' that is so popular in Saudi. The only snag is that the business is so costly that even its ordinary milk has to sell at about £1 a litre.

Begun with some assistance from Alfa-Laval, the Al Safi Dairy Farm is one of the numerous businesses of Prince Abdullah Al Faisal, eldest son of the late King Faisal. It is close to the town of al-Kharj, in a region that has been so effectively greened in the past few years that Saudi now exports wheat rather than importing most of it. What has made the transformation, of course, were water and irrigation.

As it happens, there is no real shortage of water. "Drill anywhere in the region", says one Briton employed on the farm, "and there is a 99 per cent chance of finding water." The problem is that most of the water has around 2,500 parts per million of salt, resulting in kidney problems for the cows, a milk yield that is probably considerably less than it would otherwise be, and the need for saline-tolerant crops.

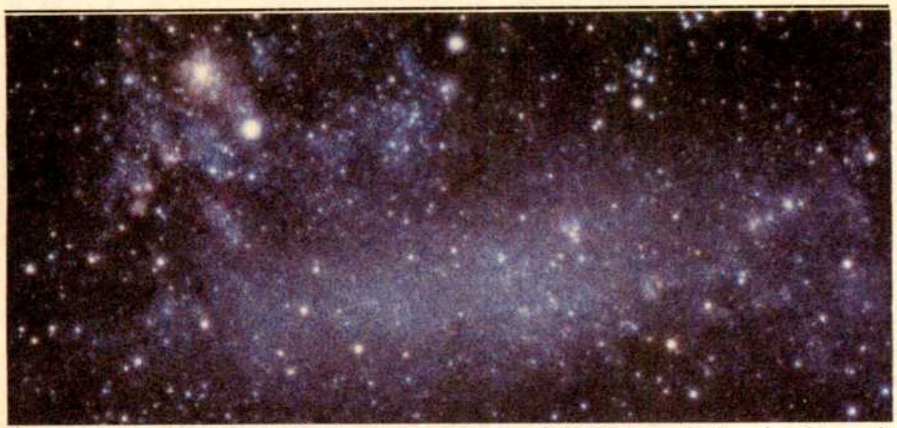
With very little, and no reliable, rainfall, the 5,000 m^3 of water needed each day comes from wells. But the original aquifer is drying out, necessitating wells to an aquifer at about 1,800 m. Not only is that water too salty to be potable but it also has to be cooled from 75°C to below 40°C before it can be sprayed on crops.

The water is distributed continuously to each field by a pivot irrigation system, similar to those responsible for the large circular fields seen from the air over parts of the United States. Around fifty 100-acre fields are cultivated in this way, growing mainly alfalfa, which can be cropped every 15 days and is used both as green feed and as a source of hay. Oats and sorghum are two other major crops.


...and in a few years, we'll have our own butter mountain!
KIRCH

The farm is impressive in its scale and integration and its water supply seems assured. But the cost and quality of the water, and doubts about the ability of the land to continue supporting such intensively grown crops, raise questions about the long-term success of the project.

Peter Newmark



THIS colour photograph of the recent supernova (SN 1987a) in the Large Magellanic Cloud (LMC) (see p. 239) was taken at the European Southern Observatory in La Silla, Chile on 25 February, two days after the supernova exploded. The supernova, which had reached a visual magnitude of 4.5 at the time this photograph was taken, is the lower right (round) of the two bright objects above and to the left of the main body of the LMC. The other, more diffuse object is the Tarantula Nebula. ☐

New science minister

Munich

WEST Germany's new minister for education and science, Jürgen Möllemann, is likely to encourage the development of privately run universities that compete with state-supported higher education. Mölleman, 41, has been a paratrooper as well as an elementary school teacher. He is the first education minister from the Free Democratic Party (FDP) and one of four FDP ministers in Prime Minister Helmut Kohl's new cabinet, one more than the last cabinet.

The FDP has long been in favour of allowing market forces to operate in German higher education and has helped to establish privately funded chairs at state-run universities. But almost all the 52 private colleges and universities in West Germany offer specialized curricula not available in the state-run educational system and therefore do not offer direct competition.

Introducing a competitive element will not be easy. The costs of establishing a new university are so high that some state support is essential. The tendency has been for the German Länder (states), which share jurisdiction over higher education with the West German government, to refuse aid on the grounds that sufficient programmes are already offered in the state-run universities. S.D.

Turk researchers harassed

London

A TURKISH research ship, the *Piri Reis*, belonging to the Marine Sciences Institute of the "Ninth September" University (Izmir) has become involved in the dispute between Greece and Turkey over drilling rights in the Aegean. The *Piri Reis* has made a number of geological survey trips both in the Aegean and the Black Sea. According to a report from the Anatolia Press Agency, Greek naval vessels shadowed the *Piri Reis* when it put to sea on 28 February. On the night of 2–3 March, the agency says, a Greek "attack vessel" with all lights extinguished approached within 30 m of the *Piri Reis* and suddenly switched on all floodlights, to the alarm of the Turks. But the captain of the *Piri Reis*, Metin Kircadagl, and Dr Sukran Dirik, the head of the scientific team, declared that research would continue as long as weather conditions permitted. V.R.

Frank Press re-elected

Washington

MEMBERS of the National Academy of Sciences have re-elected Frank Press for a second six-year term as president.

Press, 62, first became president in 1981, succeeding the late Philip Handler. He has been a member of the academy since 1968. Earlier, he served as President Carter's science adviser. J.P.

Change of heart signalled in Soviet – US health agreements

Washington

For the first time since 1978, representatives of the US and Soviet Union biomedical and public health fraternity will meet to exchange ideas. The exchanges had been regular in the mid-1970s but ceased in the wake of the Soviet invasion of Afghanistan. This time, representatives of both countries will meet for five days on the campus of the National Institutes of Health (NIH) in Bethesda to put an official stamp of approval on a new list of projects.

Prospects for collaboration are brightest in three areas: cancer, heart disease and arthritis. Claud Lenfant, director of the National Heart, Lung and Blood Institute, was a member of the delegation led by NIH director James Wyngaarden last November and has been a regular visitor to the Soviet Union. Peter Fischinger, deputy director of the National Cancer Institute, also went on the visit.

Another area of interest for both countries is Lyme disease. It is named after the town where it was first identified in the United States, and Lawrence Shulman, director of the new National Institute of Arthritis and Musculoskeletal and Skin Diseases, says there is great interest to learn whether it is the same as tick-borne erythema seen in the Soviet Union. Both are associated with ticks, but the Soviet disease seems limited to a skin disorder, whereas Lyme disease is often accompanied by either acute or chronic arthritis, and sometimes both.

The thaw in biomedical relations has been under way for some time. Last fall, two separate high-level US delegations visited the Soviet Union. A month before the Wyngaarden trip, Surgeon General C. Everett Koop headed a delegation that discussed potential exchanges in the public health arena. A speech by President Reagan in 1984 first generated the impetus for the visits, but they were delayed by differences over the treatment of Andrei Sakharov and his wife Yelena Bonner.

Two joint agreements cover all biomedical and health exchanges between the United States and the Soviet Union, one for medical science in general signed in 1972 and the other for artificial heart development signed in 1974. The agreements run for five years and are automatically renewed unless either side objects.

Even during the years of decreased contact, collaborative efforts in arthritis have persisted. Last spring, the *New England Journal of Medicine* published a joint study between researchers in the two countries on penicillamine and hydroxychloroquine as treatments for juvenile rheumatoid arthritis. Earl Brewer of Baylor College of Medicine in Houston, first author on the arthritis study, says another study using oral gold therapy will be presented at a meeting this summer, and there are new plans of a study using methotrexate as a treatment. Brewer says the arthritis studies have shown that joint protocols can be made to work, and can provide useful information.

Wyngaarden hopes that the example set by the arthritis study can carry over to evaluations of cancer therapies, speeding the development of new treatments. But although some facilities in the Soviet Union are quite modern, others must get along with primitive equipment, says Wyngaarden. He also says communication both to and within the Soviet Union can be difficult. "We seem to know more about some of their institutes than they do", he says.

Windom — new hope for health?

Some studies are politically easier to attempt in the Soviet Union than others. Studying fat or salt in the Soviet diet has been acceptable, but collaborative efforts to measure alcohol consumption would not be attempted. Political sensitivity is not restricted to the Soviets. Jack Schmidt, acting deputy director of the Fogarty International Center at NIH, says Soviet charges that AIDS (acquired immune deficiency syndrome) is somehow related to US biological weapons development "could have an impact on the extent of cooperation", particularly with regard to AIDS. Soviet interest in AIDS is on the increase (see *Nature* **326**, 5; 1987).

Although US officials see the forthcoming meeting as a positive step, they would prefer that formal mechanism for collaborations were not necessary, something the Soviet Union has insisted on.

The joint committee on health will meet on 13–17 April at NIH. Robert Windom, assistant secretary for health and Professor Oleg Shchepin, deputy minister of health, will head the two delegations. The choice of Shchepin came as something of a surprise to US officials who were expecting Yuri Isakov, deputy minister of health for international cooperation, to be chosen. **Joseph Palca**

Respite in sight for India's battered frogs

New Delhi

India, the world's largest exporter of frogs' legs, has banned the trade with immediate effect. Conclusive evidence from the Ministry of Environment and Forests and the Indian Council of Medical Research that frogs play a major role in the control of agricultural pests and mosquitoes has precipitated the ban. Frogs have now been placed in Schedule 2 of the Wildlife Protection Act of 1972, giving them a special protected status.

Export of frogs' legs from India, mainly to Europe and the United States, jumped from 390 tonnes in 1950 to 4,065 tonnes in 1982. An estimated 60 million frogs are caught each year from paddy fields and are processed for export. The frog trade provides jobs for 160,000 people and earns India about $10 million in foreign exchange.

One reason for the export ban is the increase in the pest population that attacks the rice crop. Ninety per cent of frogs' food consists of agricultural pests, including caterpillars and crabs notorious for damaging rice seedlings. It has been estimated that catching frogs for export leads to the survival of 200,000 tonnes of pests and insects, thereby requiring farmers to spend more on pesticides. India imports each year pesticides worth about $100 million, several times as much as the income from frogs' legs.

Frog catching is done at night, and frog hunters cause serious damage to rice fields. The resurgence of malaria and recent epidemics of Japanese encephalitis, which killed more than 3,000 people, are also partly blamed on the export of frogs, which are thought to restrict mosquito-breeding.

The export of frogs' legs, like that of rhesus monkeys, has been a contentious issue in India for some time. The government has been trying to conserve frogs through such measures as banning export during the breeding season (May to August), restricting the size of frogs for processing and introducing a quota system for export from different regions. It has also asked the countries of the European Economic Community to stop importing frogs' legs from India. But large-scale exploitation has continued.

The total ban on export has been welcomed by those campaigning against conditions in frog-processing centres. The Indian ban is unlikely to alter restaurant menus in Western countries so long as Bangladesh and Indonesia continue to export frogs' legs. **K.S. Jayaraman**

NASA launches wind-tunnel tests by Cray-2 supercomputer

San Francisco

A MILITARY brass band played and experimental aircraft circled as NASA (National Aeronautics and Space Administration) officials in California formally unveiled on 9 March the world's most powerful supercomputer complex for designing aircraft.

At a dedication at the NASA–Ames Research Center, 40 miles south of San Francisco, NASA chiefs hailed the Numerical Aerodynamic Simulation (NAS) system as ranking in importance with the first use of a wind tunnel in 1873 by Francis Herbert Wenham and the first flight of the Wright brothers in 1903.

For NASA, the new development is, at the least, a badly needed sign that the post-Challenger space agency has not entirely lost its knack as a pathfinder in advanced engineering. Among the most formidable tasks for the new facility will be the design of the X-30 prototype for the National Aerospace Plane, a hypothetical hypersonic vehicle endorsed by the Reagan administration.

With NAS, aeronautical engineers expect for the first time to be able to derive practically relevant solutions of the hydrodynamic equations for the flow of compressible viscous fluids developed more than a century ago and known as the Navier–Stokes equations. The solutions will be used as tools in roughing out designs of aircraft whose performance overwhelm ordinary computers, and which often cannot be tested even in the most powerful wind tunnels.

The NAS system's Cray-2 supercomputer

A Cray-2 supercomputer is, for the time being, the heart of NAS. The cooled machine is at least eight times faster than machines now used in computational aerodynamics and its memory is 40 times bigger. It is not the world's only Cray-2, but it has features giving it more versatility than any other.

In a year or so, NASA officials here expect to install a machine four times as fast. Front-runners for the sale are the still-embryonic Cray-3 or Cray YMP from Cray Research Corporation.

The NAS complex is the first routinely to use "Reynolds-averaged Navier-Stokes" equations to chart in three-dimensional detail turbulent supersonic airflow over complex aircraft bodies. Its colourful animated displays can be commanded to appear at a score of workstations at Ames and at dozens of others linked by high-speed data links at other NASA centres, aerospace companies, defence laboratories and universities.

The powerful mathematics of fluid dynamics have previously been limited to highly idealized configurations, such as isolated and geometrically simple airfoils, and often to two dimensions. The Navier–Stokes equations have been called "innocuous in appearance . . . marked by insidious pitfalls", with more than 60 partial derivative terms when expressed in three dimensions. The capacity of 10^9 operations a second expected at NAS by 1989 should make it possible to calculate flow through a grid of a million points arrayed around aerodynamic shapes.

William F. Ballhaus Jr, one of the originators of NAS, now says the facility took two major gambles. One was to order the Cray-2 in 1984 when NASA approved major spending, even though the machine did not then exist. The other was to settle on the AT&T UNIX operating system, now a common protocol in managing computer networks but not when NAS was laid out. As the Ames team guessed right, the powerful machine has been easily incorporated in interactive networks among distant laboratories.

In spite of the new computer power, wind tunnels will continue to hold a vital place in aircraft design. Ballhaus says that "a computer can optimize the design, ...but to get its actual performance out to the third decimal place, we will still need to fly a real model in real air". And then, somebody must climb into a real airplane and take off in it. **Charles Petit**

Soviet Academy of Sciences make room for next generation

London

THE Soviet Academy of Sciences last week adopted a policy of "rejuvenation". In order to make room for younger talent, nobody over the age of 65 will normally be allowed to hold an administrative post in any of the institutes, laboratories or departments belonging to the academy — a ruling incidentally that bars 65-year-old Andrei Sakharov from such a job. But superannuated academy scientists will be permitted, indeed encouraged, to devote their attention to training their successors. "In the interests of preserving intellectual potential", the new post of "honorary director" of an institute will be created, and a "staff of counsellors" made up of senior members of the academy is also envisaged.

The rejuvenation policy was formally presented and approved at the annual general meeting of the academy after several months of discussion. Last October, when Academician Vitalii Ginzburg, head of the I.E. Tamm Department of Theoretical Physics at the Lebedev Physics Institute reached his 70th birthday, he published an article in the monthly *Priroda* advocating a policy of honourable retirement for academy scientists. The Soviet Academy has for years been heavily over-burdened with septuagenarians and octagenarians (in May 1985, according to Ginzburg's figures, more than 55 per cent of full members of the academy were over 70, and 68.7 per cent over the new 65-year-old limit).

The academy, as the annual general meeting made clear, faces a massive efficiency drive. All fundamental science will be put on a footing of long-term forecasting and planning — a task that, Academy Vice-President Vladimiir Kotel'nikov noted, will have to be carried out soon and with limited resources. The academy presidium has defined 135 lines of research deemed to be of "immense significance to the national economy". Information science, the synthesis of new materials and scientific instrument-making are to receive priority attention, Kotel'nikov said, as "in these spheres difficulties arise over obtaining information from capitalist countries".

Academy departments and research establishments are to become more autonomous, which will free staff of much routine administrative work, but which will doubtless entail some interim confusion.

Scientific research in general must be made more "dynamic" and, Academy President Gurii Marchuk stressed, fundamental research must not simply support but must "overtake" the demands of technology and production. There is to be a major development of science in the east of the country, with the establishment of far eastern and Urals branches, which may well mean some scientists being encouraged to move eastwards.

In such an atmosphere of change and upheaval, many senior members and employees of the academy may well find honourable retirement a welcome option. **Vera Rich**

Howard Hughes Institute and tax collectors bury the hatchet

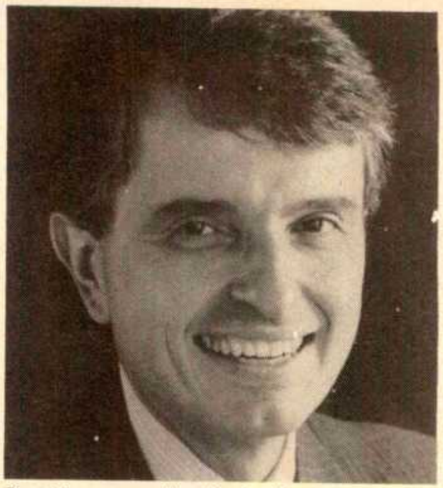
Washington

THE Trustees of the Howard Hughes Medical Institute have agreed to pay $35 million in back taxes without protest as part of the settlement of a long-standing dispute with the Internal Revenue Service (IRS) over Hughes' tax status.

In addition to the $35 million, to be paid by the end of this fiscal year, Hughes has agreed to spend $500 million over and above the minimum amount required to preserve its status as a medical research organization (MRO).

Hughes and the IRS have debated for some time exactly what MRO status means. Established in the 1950 tax code, MROs must be involved in "direct conduct of research in conjunction with hospitals", a phrase Hughes president Donald Fredrickson says has been studied "with Talmudic discipline". Very few foundations in the United States have chosen to use the MRO classification, certainly none with endowments approaching the $5,000 million of Hughes, so the rules were not clear. Fredrickson says the nature of biomedical research has shifted since 1950. Much important biomedical research is being conducted at institutions not connected with hospitals.

Hughes was anxious for the flexibility to become involved in areas not so tightly tied to clinical research. The settlement with IRS appears to make that possible. Joseph Perpich will join Hughes on 15 April to direct a new programme that will take Hughes activities into new areas of making grants and offering fellowships.



Perpich — expanding Hughes' horizons.

George Thorn, former president of the Hughes Institute and now chairman of the board of trustees, says the major emphasis will be on education. A preliminary meeting to discuss how much money is available and how it will be spent was scheduled for earlier this week in Washington.

Losing MRO status would have meant becoming a private foundation, something the institute was anxious to avoid. Foundations must adhere to more complicated rules in distributing money, and are more restricted in the type of assets they may possess, says Thorn. Private foundations must also spend 5 per cent of their endowment annually, as opposed to the 3.5 per cent required of MROs. Fredrickson says reverting to being a foundation would have required massive organizational changes in the structure of the 24 facilities the institute operates and the 1,000 people it employs. Estimating tax penalties for failing to achieve MRO status had also loomed as a potential nightmare.

Private foundations have, however, been able to survive under IRS rules. Richard Lyman, president of the Rockefeller Foundation, says he regards the 5 per cent spending figure as reasonable. Favourable economic conditions have permitted Rockefeller's endowment to grow from $1,200 million to $1,800 million this year.

The Hughes Institute's fortunes changed dramatically with the sale in 1985 of the Hughes Aircraft Company to General Motors. That sale provided the bulk of the institute's endowment, and transformed it into a major force among private philanthropies. In addition to the medical institutes it operates, and the new grant-giving endeavour, the Hughes Institute is also supporting a major effort to establish a database for human genetic information at Yale University. This new database may become the *de facto* standard for information collected by a project to sequence the entire human genome now being considered by several countries.

Joseph Palca

Sizewell B reactor gains expected approval from UK government

London

PREDICTABLY, the British government has given official approval to the construction of a £1,500-million pressurized-water reactor (PWR) at Sizewell on the Suffolk coast. The station, which will join a gas-cooled nuclear reactor on the same site, will be the first of five nuclear complexes, costing £6,000 million in total, installed around the United Kingdom.

The approval met criticism from both opposition parties; they raised doubts about the safety aspects of the PWR in the wake of Chernobyl, and the economic case for the reactor in the light of current fuel prices.

The Energy Secretary, Mr Peter Walker, was undeterred. He concluded that "there is good confidence that Sizewell B is sufficiently safe to be tolerable and that the national need for the station overrides the local interest in favour of conservation".

The reactor has been the subject of much public debate since it was first suggested in the late 1970s. A public inquiry was held between January 1983 and March 1985 from which Sir Frank Layfield, the chairman, was able to produce a report published in January of this year.

The Layfield study recommended approval, although it conceded that comprehensive tests on the safety of the design could not be carried out at this stage. The opponents of the PWR called for better assurances and claimed that the report was irrelevant because it took no account of the accident at Chernobyl.

Walker was adamant that the programme will go ahead. He said: "The PWR design for Sizewell B is of a different reactor type from the Soviet RBMK design (Chernobyl). All nuclear power stations in the UK, unlike those in the USSR, must have engineered control and automatic protection systems. Moreover, our system of regulation, unlike that which applied in the USSR, ensures that there is a proper and reliable procedural framework of controls."

The experience of the United Kingdom, he continued, demonstrated that "there is a superior safety culture" to that at Chernobyl. Many of the conservationists who gave evidence against Sizewell B do not share the minister's view and plan a public rally, to be staged in London, to protest at the government's decision. **Bill Johnstone**

Kazakh Academy scientists face party leaders' fury

London

THE former First Secretary of the Communist Party of Kazakhstan, Dinmukhamed Kunaev, whose replacement last December by an ethnic Russian, Gennadii Kolbin, triggered two days of nationalist disturbances in Alma-Ata, faces a party inquiry on charges that include corruption, nepotism and "ethnic favoritism". This is the second step in his public disgrace (he was expelled from the All-Union Politburo in January), and there is already speculation that it may end in a formal trial. Similar allegations have also been levelled aginst the scientific and scholarly community of the republic. The Minister of Higher Education, Kupzhasa Naribaev, has resigned after publicly acknowledging his faults, the leadership of the Academy of Sciences of the Kazakh SSR has been severely criticized by party leaders in Moscow, the former head of the science and education department of the Kazakh Central Committee has been severely reprimanded and a shake-up of the universities is under way.

The demonstrations began with students from Alma-Ata University taking to the streets. According to some reports, the banners carried by the demonstrators ranged from "Kazakhstan for the Kazakhs!" and "Kolbin go home!" to demands for a separate representation for Kazakhstan at the United Nations, and even for union of Kazakhstan with China.

The ethnic situation in Kazakhstan is complex. Immigration (often involuntary) from the European territories of what is now the USSR goes back to the nineteenth century, while in the 1950s the "virgin lands" policy of Nikita Khrushchev brought in a new wave of non-Kazakhs. As a result, Russians outnumber Kazakhs by 41 per cent to 36 per cent of the republic's population, with the remaining 23 per cent made up of some 20 other Soviet nationalities. But, under Naribaev, an unauthorized policy of positive discrimination in favour of ethnic Kazakhs had been in operation. Soon after the disturbances, the Soviet media noted that the student ringleaders came for the most part from southern Kazakhstan, where there is least ethnic mixing. From this area, too, came ex-minister Naribaev, and so, it appears, do six of the ten rectors of higher educational establishments in Alma-Ata.

The Academy of Sciences of the Kazakh SSR was also affected by the same failings, according to a report by the party control committee of the Central Committee in Moscow. The committee found that the academy had failed to ensure "the correct representation of scientists of different nationalities" among its members, that there was an atmosphere of "irresponsibility, obsequiousness, mutual flattery and total licence" and that the academy had wasted state funds on "ostentatious mass events", receptions, banquets and gifts to visiting notables.

Even worse, it had failed to carry out its scientific duty as coordinator of research and in the acceleration of scientific progress in the republic. Within the academy's research institutes, the control committee found, the standard of fundamental research had fallen considerably, the amount of original development work had declined, no licences had been sold and 45 per cent of the "solutions" produced turned out to be neither innovative nor useful.

None of the academy institutes had joined the new "scientific and technical complexes" in the Soviet Union to obviate bureaucratic delays in technical innovation. Nepotism and local preference had led to the appointment to senior scientific posts of poorly trained and "morally lax" people.

The main responsibility for these shortcomings was attributed by the control committee to the former president of the academy, Askar Kunaev, who was replaced last year. During his term of office, said the report, Kunaev "crudely flouted the principles of collective leadership", ignored critical observations and advice from the All-Union Academy in Moscow, drank to excess and often failed to come to work or to deliver important speeches.

Vera Rich

Super conductivity

New Delhi

A TEAM of researchers at the Tata Institute of Fundamental Research in Bombay and the Bhabha Atomic Research Centre at nearby Trombay claims to have demonstrated the onset of superconductivity in a synthetic oxide at a temperature of 120 K as well as zero electrical resistance at 90 K. The team says that it has thus surpassed the record of superconductivity at 82 K set by a group led by C.W.Chu at the University of Houston (see *Nature* 325, 756; 1987).

Although the materials used by the two groups are very similar, the Indian team claims that their success in finding a higher transition temperature arises from "sample stoichiometry, heat treatment and preparation technique". The team used samples of yttrium/strontium/barium copper oxide which were annealed in an atmosphere of oxygen and powdered and cast into disks of 8-mm diameter. A month ago, the team found zero electrical resistance in a similar compound with lanthanum instead of yttrium.

K.S.Jayaraman

SERC admits funding crisis with no respite in the years ahead

London

THE Science and Engineering Research Council (SERC) has formally admitted that the new salary increases agreed with academic staff last month will have a dramatic effect on its research grant budget. The new salaries will cost an extra £7.8 million in the year beginning 1986 and the following year will rise to £10 million or about 'one-tenth of the annual research grant expenditure'.

SERC is concerned that the full weight of these increases must be borne by its current budget. A similar shortfall of some £20 million because of the increase in the European Organization for Nuclear Research (CERN) due to currency fluctuation, provoked the council to apply this year to the Advisory Board for the Research Councils (ABRC) for extra funds.

The disclosures are outlined in the council's new three-year plan, *The forward Look, 1988/89 to 1990/91* published last week, which concludes that: "There are important areas of basic and strategic work which Council will not be able adequately to exploit".

The announcement of the SERC shortfall coincides with statements made by the organization called Save British Science which has called government attention to a deepening crisis within the research councils. Scientists are uncertain, they claim, whether their current applications will even be considered, and are fearful that their work will be "arbitrarily terminated".

Meanwhile, a working group commissioned by the government will advise on applications of defence research and development to the commercial sector.

Bill Johnstone

Hocus pocus

THERE seems to be in wide circulation a letter purporting to be from *Nature* and referring to a paper entitled "Neoplasms as uncontrolled regenerative hyperplasia" by Cris Garcia Sarmiento (deceased).

We should like to make it clear that this letter is a hoax, that we have no knowledge of either the paper or the author, and that other organizations as well as ourselves have been victims of this hoaxer.

Editor, *Nature*.

Problems of Portuguese science

SIR—As a Portuguese scientist working for twelve years in West Germany and associated with the academic society of Portugal for five, I read the survey on Science in Iberia (*Nature* **324,** 313–332; 1986) with amusement: your journal has been used very effectively for the international airing of proposed programmes of the Portuguese Ministry of Education and its institutions. Whether these programmes will ever become a reality only time will tell.

I do not wish to question the good intentions of those concerned, but their effectiveness. This government has been in office for over a year. The real problems of science in Portugal are: (1) the legal structure of its institutions and their hiring policy (people are rarely fired) and (2) the attitudes and scientific competence of the majority of people in these institutions.

The first problem concerns the ministry and its power to introduce reforms. Why, for example, do teaching assistants with the equivalent of a master's degree effectively have tenure and a guaranteed permanence in the educational system? Why is there so much inbreeding in Portuguese educational institutions? Why are academic positions in Portuguese institutions almost never advertised in the international scientific press, where they would be brought to the attention of interested and qualified Portuguese living abroad? Why is it possible for Portuguese academics to have more than one job?

The second problem has historical roots which have both affected the Portuguese attitude towards science and the world and filled the Portuguese institutions with an astonishing number of incompetent people. It is well known that any multistep process, such as an educational or scientific establishment, is only as efficient as its least efficient step. There are some excellent and internationally competitive people and research groups in Portugal, but they are relatively few. Progress is, more often than not, hindered by the almost uncountably large number of dog-in-the-manger types who still prevent the opening of Portuguese scientific culture to the rest of the world. This is partly because they feel intimidated by science at a modern international level and partly because they no longer speak the same language. Admittedly, the Ministry of Education is not directly to blame, but some initiative for change should, nevertheless, come from there. Why, for instance, is there no legislation enforcing a periodic evaluation of scientific and teaching competence of Portuguese scientists and professors as there is in almost every other progressive country? Why is demonstrated excellence never materially rewarded? Why are students in Portuguese universities never asked to evaluate course or entire degree programmes offered to them?

Finally, some specific comments regarding statements in your survey: Every Portuguese is very used to hearing catchy terms like "new blood" (p. 332), which makes it all the more difficult to understand why inbreeding is still a way of life in Portuguese institutions of higher education. "Patience" (p. 330) with all officialdom in Portugal is a necessary requisite for survival in that country. It is not the authorities that require this commodity.

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Letters submitted for Correspondence should be typed, double-spaced, on one side of the paper only. □

Citation counts

SIR—The recently reopened debate about citation counts (*Nature* **324**, 95; 1986 and **325**, 478; 1987) has highlighted the fact that we still do not know enough about citation behaviour to endorse the use of such analysis without qualification in matters of resource allocation. The technique aims at quantifying the impact of published research, this being one measure of the research output of university departments. Output should not, and cannot meaningfully, be considered in isolation from input, however.

A recent study conducted at Lancaster has investigated the determinants of research output in departments of economics in 40 British universities. A bibliometric measure of output per staff member (call it X_1) was constructed for the years 1980–84. This was expressed as a function of university and department size, staff age, staff–student ratio, library stock, external funding and numbers of professors and research students. More than 70 per cent of the variation in X_1 can be explained in this way. The regression residuals — which represent that part of output that cannot be explained by differences in the inputs defined above — could themselves conceivably be used as a performance indicator. Call this latter measure X_2.

The Spearman's rank correlation coefficient obtained by comparing X_1 and X_2 is −0.597. This indicates no significant degree of positive correlation between the two measures. If this is the case for economics, one shudders to think at how misleading citation counts might be for sciences where resource costs are greater.

Presumably the aim of performance indicators is to estimate value added. Citation counts alone are quite incapable of doing this. Even if bibliometric analysis could crudely reflect research output, they alone could never measure productivity.

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New Zealand denial

SIR—In an article "Genetic manipulation living outside regulation" (*Nature* **324,** 202; 1986), it is stated that a team from Oregon State University in Corvallis had field-tested a modified vaccinia virus in sheep, cattle and chickens in New Zealand. The facts are that a scientist from Oregon State University came to New Zealand and requested permission for a field test of the vaccinia/sindbis oganism, but this request was denied. After consideration of the request and careful examination of the experimental protocol by the Wallaceville Animal Research Centre Biological Society Committee, in consultation with the New Zealand Advisory Committee on Novel Genetic Techniques, tests were allowed in calves and chickens under strict quarantine. No field trials were permitted and as far as I am aware there have so far been no field trials of any genetically engineered organisms in New Zealand.

J.N. PARLE
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University rows

SIR—I read with interest your opinion on professional athleticism at Oxford (*Nature* **325,** 470, 1987).

The boat race between the eights of the Universities of Pavia and Pisa takes place each year alternately on the waters of the Ticino and of the Arno, the rivers flowing through the two old university towns. The rule that the rowing men must be students has in the past caused a hiatus of some years in the annual event when the presence in both crews of oarsmen who would not know what the social sciences were was considered too much, even given the Italian genius for compromise.

We did not at the time indulge in the luxury of brooding on such trivial matters but the survival of the two universities was then as always threatened by lack of funds. The University of Oxford is to be envied for being able to enjoy such luxuries when short of funds.

MARCO FRACCARO
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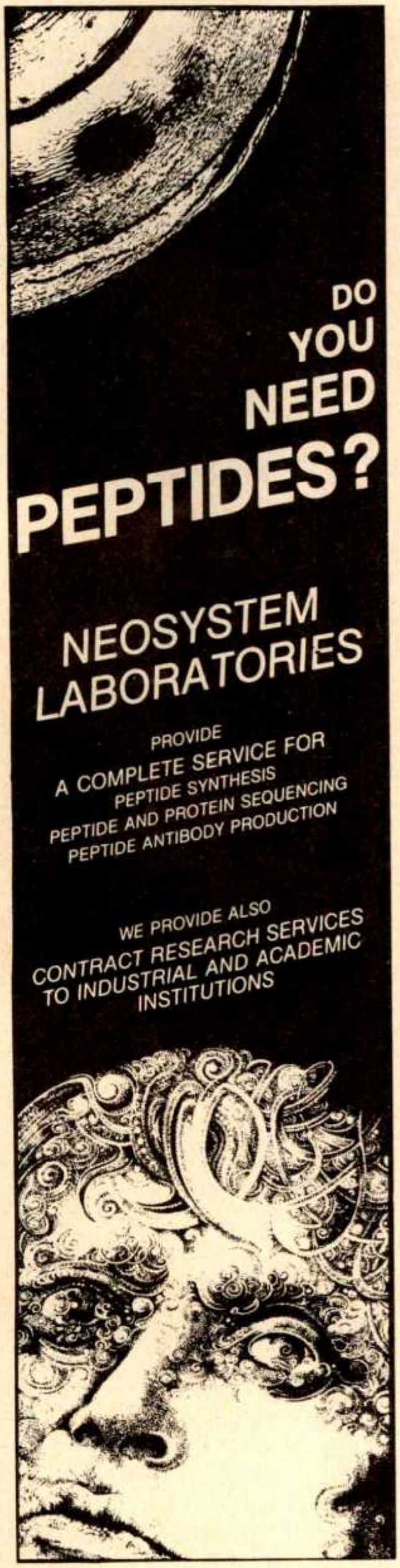

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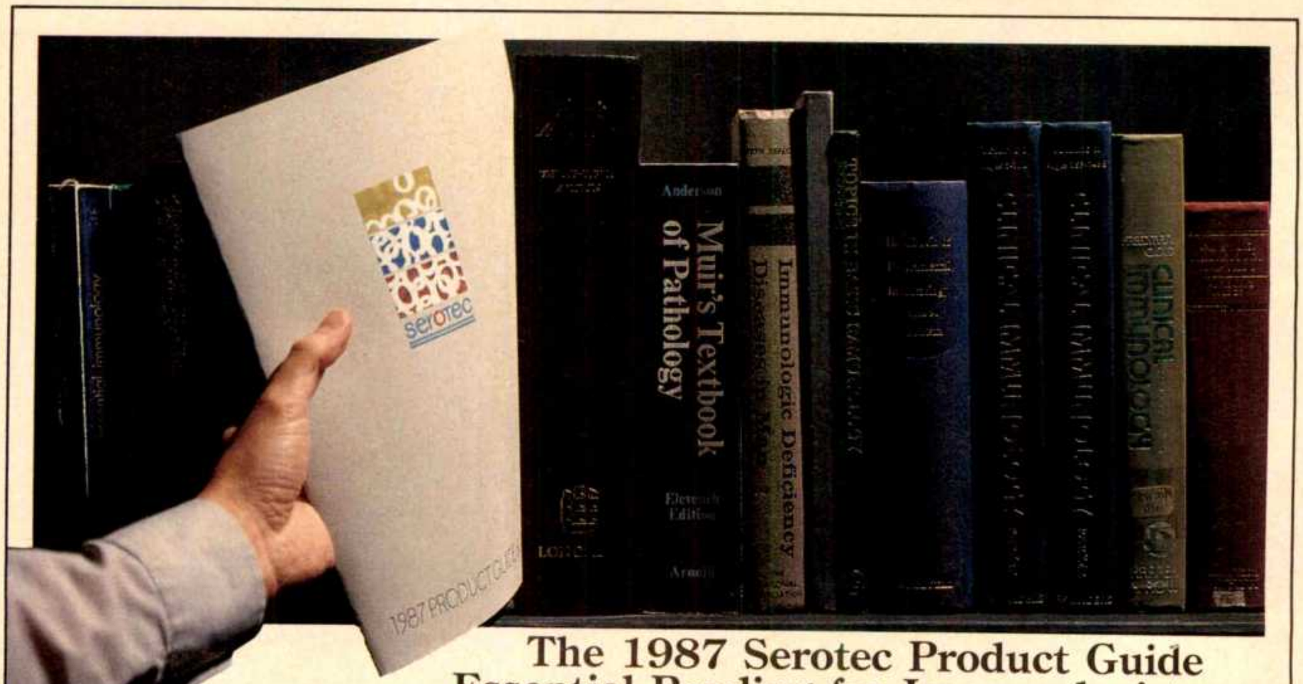

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Neutrinos from the supernova

Physicists appear to have reacted as promptly as the better-drilled astronomers to the opportunity presented by the explosion of a supernova in the Large Magellanic Cloud on 23 February.

THE first few weeks of Supernova 1987a have seen a flurry of IAU (International Astronomical Union) telegrams, hastily written preprints and late night telephone calls. Astronomers, already accustomed to dealing with unexpected celestial events, have mobilized with admirable speed. It is even more remarkable that physicists at underground detectors around the world have reacted with equal speed to the reported detection of neutrinos from the explosion. Now, as the supernova itself continues to shine steadily in the Large Magellanic Cloud (LMC), some of the confusion of the first few days is being dispelled.

The detection of neutrinos has created much excitement. The announcement by the Italian experiment in the Mont Blanc tunnel that neutrinos had been seen was soon followed by a conflicting report from Japan. Physicists at the Kamioka zinc mine also saw a burst of neutrinos, but four and a half hours later than the Mont Blanc events. Because a supernova can emit neutrinos only at the very instant of explosion, these reports could not both be right. Yet at the outset it was thought that no other experiment would be able to see the neutrinos (see *Nature* **326,** 11; 1987). In particular, the high-threshold IMB (Irvine–Michigan–Brookhaven) device, situated in an Ohio salt mine, can see neutrinos only above 20 MeV, where the thermal spectrum of neutrinos from a supernova explosion should produce almost nothing.

While the discordant observations from Italy and Japan were being pondered, the IMB collaboration announced that it had in fact seen neutrinos from the supernova, and with a clear and unambiguous signal. Starting at UT 7:35:41.37 on 23 February, eight events at an energy between 20 and 40 MeV were recorded over a period of six seconds. To within a few seconds, this is the time the neutrinos arrived in Japan, but as the Kamioka experiment does not have accurate absolute timing, the two can be regarded as simultaneous.

Although this agreement was gratifying, there was a problem. Kamioka saw just one event at 35 MeV, while IMB saw eight events of comparable energy. After a little thought, this turned out not to be a difficulty. A supernova does indeed produce fewer neutrinos at higher energy, but this is partly compensated for by the increase of neutrino interaction cross-section with the square of the energy: high-energy neutrinos are therefore more likely to be detected and for these particular neutrinos, the IMB detector is effectively seven times more sensitive than that at Kamioka.

In all this excitement, the Italian detection has rather been forgotten. The general opinion is that it saw a chance cluster of events, only a little above background noise. At both IMB and Kamioka, data have been re-examined at the time of the supposed Italian events; nothing has been found.

Meanwhile, as the euphoria of this first achievement of extragalactic neutrino astronomy subsides, physicists are trying to extract more subtle information from their data. Theoretically, at the moment of core collapse, a supernova will emit a burst of neutrinos lasting for about a second. Yet IMB and Kamioka report neutrino events over periods of six and thirteen seconds, respectively.

One explanation is that neutrinos have mass: massive neutrinos travel at less than the speed of light, so that the arrival times of neutrinos of different energy would be spread out. A simple estimate suggested that a mass of some tens of electron volts would disperse the neutrinos over a period of six seconds during their passage from the LMC. But there is a more prosaic explanation. Rather than coming directly to us from the supernova's collapsed core, the neutrinos would most probably have been scattered a few times by atoms in the stellar atmosphere on the way out, their arrival delayed by a few seconds.

In principle, the effects of neutrino mass and simple scattering can be distinguished by careful study of the energy spectrum and arrival times of the neutrinos, because the two effects depend differently on energy. People at IMB and Kamioka hope that their data will shed some light on this question, but it is unclear whether the neutrinos detected worldwide are enough to establish any useful spectral information.

The mere detection of neutrinos is proper cause for jubilation, but astronomers have been in this business longer and have more exacting standards. They continue to build up their picture of the supernova, comparing that with those of previous supernovae observed in galaxies more distant than the LMC. According to Robert Kirshner, of the Harvard–Smithsonian Center for Astrophysics, SN 1987a must be categorized as Type II because it has hydrogen lines in its emission spectrum, but is not altogether typical.

SN 1987a is fainter than one would expect, its light output rose more steeply than usual and has then quickly levelled out. What will happen next is not quite clear. The light curve of a typical Type II supernova rises to a maximum and then begins to fall in a matter of days. But SN 1987a does somewhat resemble two supernovae observed in the 1940s, which, after a plateau of constant brightness, brightened again before subsiding.

Different Type II supernovae may behave differently because the early and late periods of the light curve are differently controlled. The luminosity of the initial outburst derives from the mechanical energy of the explosion, but in the later stages the energy source is the decay of radioactive nuclei, especially isotopes of cobalt synthesized in the explosion. Unusual ups and downs in the light curve may thus reveal something about the original explosion, the progress of nuclear fusion in the outburst, and the chemical composition of the progenitor star.

This progenitor has not yet been identified. The star Sanduleak-69.202, close to the explosion site, was the first and would have been the ideal candidate — except that observations by the International Ultraviolet Explorer satellite show that it is still there, as indeed are all the known stars in the vicinity. But Kirshner is not greatly concerned. He believes that the progenitor was a slightly fainter star, near the line of sight to 69.202 and obscured by it. Even so, it is irksome that the first nearby supernova for several centuries should have another star right in front of it.

SN 1987a may nevertheless be cosmologically important. It is close enough for Kirshner to hope that speckle interferometry will reveal the angular size of the expanding shell over the coming weeks and months. Knowing the brightness, expansion speed, size and distance, it will be possible accurately to reconstruct the explosion and the subsequent expansion, which in turn will help similarly to reconstruct supernovae in more distant galaxies. Because careful observations of extragalactic supernovae can provide good estimates of their distance, the potential payoff is that SN1987a will help to fix more accurately a few points on the still sparsely occupied plot of galactic redshift against distance.

David Lindley

Lesch–Nyhan syndrome

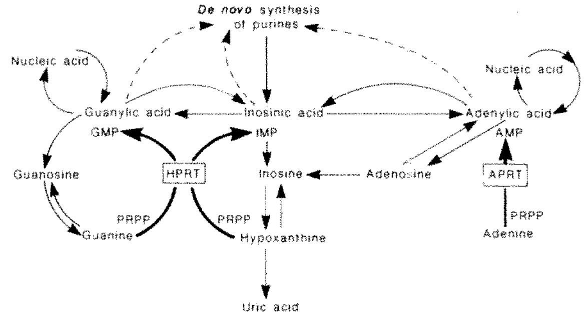
Engineering mutant mice

Brigid Hogan

LESCH–NYHAN syndrome is a rare, tragic inherited disorder affecting boys[1]. The major symptoms are spasticity of the limbs, involuntary movements, mental retardation and the bizarre habit of compulsive self-mutilation. It is classic, textbook knowledge that the syndrome is caused by deletions and rearrangments in the X-linked gene encoding hypoxanthine phosphoribosyltransferase (HPRT), an enzyme in the 'salvage' pathway for recycling purine nucleotides (see figure). But although the Lesch–Nyhan syndrome is well understood in terms of molecular genetics[2], surprisingly little is known about how the neurological symptoms develop, and schemes for alleviating or preventing them by some form of somatic cell gene therapy are largely speculative[3]. To help overcome these problems, people have been searching for an experimental model of the Lesch–Nyhan syndrome produced either by a spontaneous mutation in a laboratory animal or by specifically engineering a completely defective *Hprt* gene into the germ line of mice. On pages 292 and 295 of this issue, Hooper *et al.*[4] and Kuehn *et al.*[5] independently report success in generating HPRT$^-$ male mice by injecting into normal embryos pluripotential stem cells (ES or EK cells) which had first been selected as HPRT$^-$ in tissue culture.

The results show that the injected cells mingle with the normal embryo cells and contribute to all the differentiated tissues of the chimaeric adult. Gametes derived from the ES cells then pass on the X-linked *Hprt*$^-$ gene to the next generation. Although, as I shall explain, it is too early to judge exactly how useful the HPRT$^-$ male offspring will be as a model for the human disease, the two papers undoubtedly represent a milestone in mammalian genetics. They clearly establish the feasibility of using ES cells for introducing selected mutations into the germ line of mice and for studying their effect on the whole organism. Moreover, they provide new impetus for overcoming the final hurdle of replacing a normal gene with a specifically engineered mutant version by homologous recombination, an achievement that would be a major advance in mammalian developmental genetics.

Apart from the medical interest of the Lesch–Nyhan syndrome, there are several reasons why *Hprt* is an ideal gene for routing mutations into the germ line. First, it is expressed by all cells, and only one copy needs to be knocked out in XY cell lines to eliminate enzyme activity completely. Second, the gene presents a reasonable target size (34 kilobases) and cloned probes enable the sites of mutation to be mapped. Third, and most important, a powerful technique is available for selecting HPRT$^-$ cells, which are unable to salvage free purine bases. Therefore, unlike HPRT$^+$ cells, they are not killed by toxic purine analogues such as 6-thioguanine and 8-azaguanine added to the culture medium. This selection technique was used by both groups[4,5] to isolate HPRT$^-$ stem cells, but the strategies behind generating the mutants were rather different, and reflect the present diversity of interest in embryonic stem cells.



Simplified scheme for purine metabolism. APRT, adenine phosphoribosyltransferase; PRPP, 5-phosphoribosyl-1-pyrophosphate; dashed lines, feedback inhibition. Reduced levels of HPRT activity lead to intracellular loss of hypoxanthine and an increased *de novo* synthesis of purines, caused by release of feedback inhibition. The hypoxanthine is oxidized to uric acid, which in humans builds up in the blood and urine. The neurological symptoms of Lesch–Nyhan syndrome develop when HPRT activity is below about 1 per cent of normal, presumably because of the toxic effect of abnormal levels of some intermediary metabolite.

One of the long-term goals of Martin Evans and colleagues in Cambridge is to use retroviral infection of ES cells to generate insertional mutations in a wide range of mouse genes, and so provide a valuable resource for mammalian genetics[6]. The technique for retroviral insertion described by his group in this issue[5] involves replacing the feeder cells that are normally used to maintain their XY stem cells in an undifferentiated state with fibroblasts that release a disabled retroviral vector. During the incubation period about 20 viruses are taken up by each stem cell and inserted into the chromosomes at different sites. The cells are then returned to normal feeder cells and selected for resistance to 6-thioguanine. The few surviving HPRT$^-$ colonies are cloned and assayed for insertion of a retrovirus into the *Hprt* gene by Southern blot analysis of isolated DNA using a complementary DNA probe. The mouse *Hprt* gene, for example, does not contain sequences recognized by the restriction enzyme *Kpn*I, but these sequences are present in the virus. Viral insertion therefore introduces *Kpn*I cutting sites into the *Hprt* gene. It also presumably disrupts either elongation or normal splicing of the *Hprt* gene transcript *in vivo*, resulting in failure to produce mature messenger RNA. After selection, the stem cells are injected into host blastocysts of a different genotype and implanted into foster mothers. Offspring that have incorporated the injected cells are recognized by their coat colour chimaerism and are test-bred with normal mice. In one case the original XY stem cells had lost the Y chromosome during selection and were therefore XO. However, these cells can successfully make oocytes so that the chimaeric female passed on the *Hprt*$^-$ gene to its offspring when mated with a normal male, producing both HPRT$^-$ hemizygous XY males, and HPRT$^-$/HPRT$^+$ heterozygous, XX, 'carrier' females. One potential drawback of this technique is that the stem cells contain on average more than one inserted retrovirus, so that it is theoretically possible, although statistically very unlikely, that a second X-linked gene has been disrupted. By continued crossing with normal mice it is possible selectively to breed out the other viral insertions and to test them for possible recessive mutations in autosomal genes.

The strategy for selecting HPRT$^-$ stem cells described by Hooper *et al.* in this issue[4] was somewhat different. This group, at the University of Edinburgh, has developed a method for maintaining ES cells in the undifferentiated state in the absence of feeder fibroblasts using conditioned medium from buffalo rat liver cells[7]. This procedure makes culture and selection of the stem cells much easier, and also opens up the possibility of identifying factors required both for the growth of the stem cells and for the regulation of their differentiation. By adding 6-thioguanine to the conditioned medium of an XY cell line the authors selected spontaneous HPRT$^-$ mutants already present in the population. One of these was used to make germline chimaeras, in collaboration with Alan Handyside and colleagues at the MRC Experimental Embryology and Teratology Unit in Carshalton, and with Marilyn Monk at the MRC Mammalian Development Unit in London, who has developed microscale methods for measuring HPRT activity in single embryos and hair follicles. The pattern of inheritance of the *Hprt*$^-$ gene from the founder chimaeras is essentially the same as that reported by Kuehn *et al.*. Crossing an HPRT$^-$/HPRT$^+$ carrier female with a normal male generates both HPRT^- male, and normal and heterozygous female embryos, all of which can be distinguished by enzyme assay at the morula stage. This augers well for early prenatal diagnosis of HPRT^- XY human embryos by measuring enzyme activity in single blastomeres sampled from 8- or 16-cell morulae.

The male HPRT^- mice generated by both groups are anxiously being watched for signs of behavioural abnormality, but so far they appear perfectly normal. Current ideas about the origin of the neurological symptoms in Lesch–Nyhan humans centre around the observation that HPRT activity is about five times higher in certain regions of the brain (the basal ganglia) than in other cells. Absence of HPRT activity in the basal ganglia appears to be associated with poor development of the terminal arborizations of dopaminergic neurons. It may be just too early to see symptoms in the HPRT^- mice, because in humans the most dramatic effects do not develop until several months after birth. Alternatively, there may be subtle differences in purine or neurotransmitter intermediary metabolism between mice and humans which means that mouse neurons escape damage. Pinpointing these differences could lead to the design of new drugs. Meanwhile, clever schemes are being devised for selecting other mutations in X-linked and autosomal genes expressed in ES cells, and for rapid screening of pools of infected cells for retroviral insertion into genes for which selection is not so simple. The full potential of the technique will be realized with the development of methods for replacing normal endogenous genes with specific mutations by homologous recombination. □

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Natural selection

Competition among different taxa

Jared M. Diamond

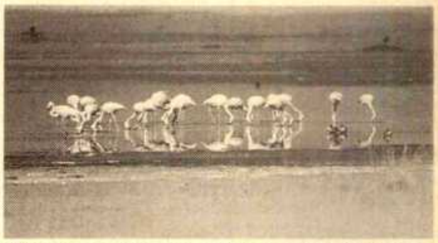
As species of the same genus have usually, though by no means invariably, some similarity in habits and constitution, and always in structure, the struggle will generally be more severe between species of the same genus, when they come into competition with each other, than between species of distant genera[1].

FOLLOWING this opinion of Darwin, ecologists have tended to focus on competition between closely related species, especially members of the same genus. But a critical resource can also be shared by taxonomically remote species. Antarctic krill, for example, sustain baleen whales, seals, penguins, petrels, fish and squid, not to mention krill-harvesting ships. Thus, competition between divergent taxa could be significant. Recent studies pose questions about how taxonomic closeness affects the consequences of competition.

Inferences of competition can be based either on indirect evidence (such as shared limiting resources and complementary spatial and temporal distributions) or on direct evidence (such as observations of aggression and effects of removal experiments). These types of evidence are illustrated by Carpenter's field studies[2] of Californian flowers whose nectar is sometimes in short supply and on which hummingbirds, bees, moths, butterflies and flies depend. Hummingbirds chase bees and moths from flowers, whereas bees in turn chase hummingbirds. When hummingbirds are present, visits to flowers by moths decline because hummingbirds chase some moths away and interrupt the foraging bouts of the rest. Moth visits peak at noon in the absence of the birds, and at dawn or dusk in their presence (hummingbirds forage during the day). When Carpenter enclosed flowers in cages that excluded hummingbirds but let bees in, visits to the flowers by bees became more frequent and lasted longer.

Birds and bees are also the subject of a recent study[3] by Schluter on the effects of competition between bees and Darwin's finches visiting flowers in the Galapagos Islands. Finches spend 20 per cent of their time collecting nectar on islands lacking bees, but only 4 per cent of their time on islands with bees. In the absence of bees the finches shift in body size towards the smaller bees, from weights of 14 – 21 g on bee islands to 10 – 12 g on islands without bees. On the islands without bees, individual finches that visit flowers are smaller (average weight 8.7 g) than individuals not visiting flowers (11.3 g). The explanation is that nectar feeding is more economical for bees and small birds than for large birds, because they thereby satisfy a higher fraction of their energy needs.

Hurlbert *et al.* have recently obtained evidence[4] that flamingoes compete with fish for zooplankton at high-altitude lakes in the Peruvian Andes. The number of flamingoes per lake varies from 0 to 4,457. Fish predation on zooplankton reduces zooplankton biomass ninefold in lakes with fish compared with lakes without fish, and shifts the zooplankton composition from large-bodied to small-bodied species. As a result, flamingo numbers are 10 times lower at lakes with fish than at lakes without fish. Most of the instances of lakes entirely without flamingoes turn out to be lakes with fish.



Flamingoes at Laguna Parinacochas, Peru, feed on the abundant zooplankton in the absence of fish. (Courtesy of Stuart Hurlbert.)

In the light of such studies, how might competition between distant taxa differ from that between members of a genus? Competing members of a genus often exhibit mutual aggression; mutual displacement of each other in space and time; and mutual character displacement (evolutionary changes) in morphology in each other's absence. The more dissimilar the competitors, the more asymmetrical the three phenomena are likely to be. Hummingbirds, for example, chase moths and not vice versa, and the sheer numbers of the smaller moths may swamp the birds by forcing them to devote so much time to chasing that foraging becomes uneconomical ('aggressive neglect'; see ref. 5). Trapping seed-eating rodents causes a much greater increase in abundance of seed-eating ants than the increase in abundance of rodents caused by poisoning the ants[6].

The generality of these predictions requires further testing. They gain interest because of the possible economic importance of competition between humans and other animal species and the implications for conservation. Thus, hunters blame wolves for depleting deer and fishermen blame sea birds for depleting fish. More likely, however, the asymmetry of competition is in our favour: human starvation caused by effects of sea birds has yet to be documented, but massive starvation of sea birds has certainly resulted from overfishing by humans[7]. □

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Intracellular signalling

Receptor-operated Ca channels

Erwin Neher

THERE is hardly any tissue where receptor-operated calcium channels have not been postulated. Evidence from biochemical and tracer flux studies indicates that they are involved in many diverse cellular functions (see ref. 1 for a review). In contrast, electrophysiologists have had until recently only one well-characterized mechanism to explain receptor-mediated changes in Ca conductance: the second-messenger-induced modulation of voltage-activated Ca channels, which has been found in several systems[2-4]. No such voltage-activated channels, however, have been found in cell types such as mast cells[5] that are believed to possess receptor-operated Ca channels. But in the past few months, three more mechanisms of receptor-mediated Ca flow have been suggested. One is described by Kuno, Gardner and colleagues (ref. 6 and page 301 of this issue[7]); another was reported[8] by Reuter and colleagues; and a third was proposed[9] by Irvine and Moor.

Inositol (1,4,5) trisphosphate (InsP$_3$) is well established as a second messenger that releases calcium from intracellular stores[10,11]. The report in this issue[7] describes a second, indepedent effect of InsP$_3$ on calcium influx. In T lymphocytes, the authors observe current fluctuations resulting from opening and closing of very small, Ca-permeable channels directly activated by intracellular InsP$_3$. In their earlier work[6], they observed a very similar channel in human T lymphocytes after activation by mitogen. The channel is not or is only marginally voltage-dependent and is blocked rather than activated by increasing calcium concentration, $[Ca]_i$, on its cytoplasmic side. The opening of this channel therefore cannot be caused by increased $[Ca]_i$, which shows that this is not an indirect consequence of Ca release from intracellular stores.

Such channels, which mediate what might be called a Ca-induced Ca influx, have recently been described[8] by Reuter and colleagues. These authors observed a rise in $[Ca]_i$ following peptide-induced neutrophil activation and the consequent opening of two types of nonspecific channels that are permeable to Ca and other cations. These channels are opened only by increased $[Ca]_i$ and not by InsP$_3$ or by the peptide directly. This mechanism of Ca-mediated activation of Ca-conducting channels could be a widespread phenomenon that is used by cells to perpetuate an InsP$_3$-induced increase in $[Ca]_i$, which by itself is only transient.

The third mechanism of receptor-mediated calcium inflow through the plasma membrane which has recently emerged also involves InsP$_3$. Irvine and Moor have shown[9] that inositol (1,3,4,5)tetrakisphosphate (InsP$_4$), derived from InsP$_3$ by phosphorylation, may serve as a messenger on its own (see the recent discussion in News and Views[12]). When injected into sea urchin eggs, InsP$_4$ causes a raising of the fertilization envelope, a process which is dependent on the presence of extracellular Ca. This result suggests an InsP$_4$-activated channel.

The three mechanisms described (Ca-permeable channels directly opened by InsP$_3$; channels opened by an initial InsP$_3$-induced increase in $[Ca]_i$; and putative channels opened by InsP$_4$) will probably help to elucidate the role of calcium in many diverse cellular responses. It is now becoming clear that a phase of sustained increase in $[Ca]_i$ dependent on external Ca can be separated from a transient phase independent of external Ca (mediated by InsP$_3$) in many types of electrically inexcitable cells. The channels described here have properties suggesting that they mediate such a sustained phase. Although they probably are the receptor-operated Ca channels that have long been searched for, they are not Ca channels in the traditional sense. They lack ionic specificity, so that under physiological conditions the ion contributing most to the current is not Ca.

At first glance one is reluctant to accept three new mechanisms. But only a limited body of data is available at the moment. It will be interesting to see how many separate mechanisms will remain once they are more completely characterized. It is conceivable that the effect of InsP$_3$ on lymphocytes, which now seems to be a direct action of InsP$_3$, is in fact caused by InsP$_4$ generated by the cell from InsP$_3$. This possibility is considered by Kuno and Gardner[7]. On the other hand, Irvine and Moor mention[9] that the action of InsP$_4$ is enhanced by prior Ca mobilization. This may provide a link to the channels described[8] by Reuter and colleagues. ☐

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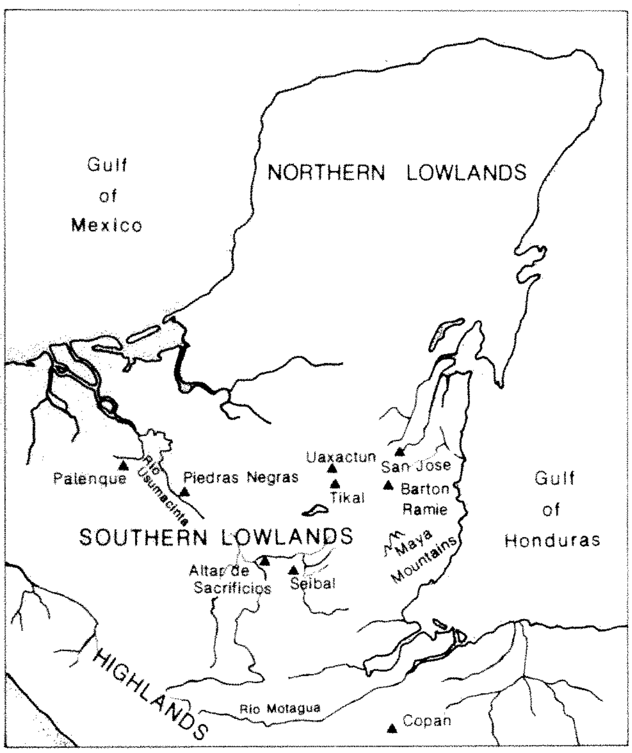
Archaeology

New perspectives on the history of ancient Maya civilization

Jeremy A. Sabloff

OUR understanding of ancient Maya civilization has undergone huge changes in recent years. Although the exciting breakthroughs in deciphering Maya hieroglyphic writing and in appreciating the ideological roles of the Maya elite have justifiably received much publicity[1], equally significant breakthroughs have been made in understanding the agricultural and adaptive foundation upon which Maya civilization was built[2,3]. All these advances have caused significant changes in traditionally held views of the rise, development, and so-called 'collapse' of Classic Maya civilization. Thus, in contrast to older views, Classic civilization is now thought to have been composed of urban centres, supported by both extensive and intensive agriculture, closely linked economically, politically and ideologically, and subject to widespread raiding and conflict. Whereas Mayan civilization was considered unique, it is now believed to have developed in a similar manner to other pre-industrial complex societies. The new pollen data presented by David Rue on page 285 of this issue[4] should lead to further revisions of the traditional picture. Assuming Rue's interpretation of the pollen in his two samples is correct — and it appears both plausible and well argued — some re-examination of current views is called for.

For many years, the earliest Maya remains of settled farmers could not be securely dated before about 800 BC. Although many suspected that earlier remains existed, there were no data to support such suspicions until the major discoveries[5] by Norman Hammond at the site of Cuello in Belize, which pushed this date back about 1,500 years to 2300 BC. Hammond has argued that the occupants of Cuello in the late third millennium BC were settled, pottery-making, maize agriculturalists who culturally were Maya. But it remains an open question whether the occupants of Cuello migrated there from outside the Maya lowlands, bringing along with them their agricultural and ceramic skills, or whether they had a long lowland history as well as one of agriculture and pottery-making. Richard MacNeish's recent work in Belize led him to argue[6] for a long, pre-ceramic, lowland occupation.



Rue's new pollen-core data from western Honduras described in this issue[4] broaden our understanding of this question. The data provide evidence for slash-and-burn agriculture, probably part of a hunter–gatherer strategy. Given the current lack of ceramics dated to pre-800 BC in western Honduras, Rue's results suggest that there were pre-ceramic peoples practising agriculture there as far back as the fourth millennium BC. This finding indicates that agriculture existed long before the rise of complex societies in the lowlands and did not have a much shorter history of development, as previously believed. To confirm this result, it is necessary to find archaeological remains that can be directly tied to Rue's pollen data. Such evidence will elucidate the nature of this early agricultural occupation, its relation to sedentary and/or mobile adaptations and its possible links with later fully sedentary, village agriculturalists. It is to be hoped that fieldwork directed to this end will soon begin both around Lake Yojoa, near Palenque and elsewhere in the lowlands (see map).

Moving from the foundation of Maya civilization towards its later stages, the 'collapse' of the Classic Maya has also been the focus of much attention. Recent research indicates that earlier notions of cultural collapse and demographic abandonment in the southern lowlands are not tenable, and that some sites and regions were not abandoned or were only partially depopulated. Furthermore, there were significant demographic movements and shifts of economic and political centres during the ninth century AD (refs 7,8). Rue's pollen discoveries make a useful contribution to the consideration of the demographic and cultural changes at this crucial time by indicating first, that there was very widespread clearing of forest cover in the region around the great Classic Maya city of Copan (see map) at the time of the collapse of this centre; and second, that there was occupation in this region for several centuries after the abandonment of Copan.

First, the pollen evidence for major forest clearance when Copan stopped being an urban centre will support the position[9] that ecological stress and soil degradation were important in the downfall of many centres in the southern lowlands during the ninth century AD. Although such degradation may not have been present around all large sites and was probably not the sole cause for the abandonment of many centres, ecological catastrophe at certain key sites could have led to disruptions among centres with close economic and political ties. This could have made cities in the northern lowlands, such as Uxmal, Sayil and Chichen Itza, which began to flower at this time, much more attractive to the Classic Maya population in the south and to non-Classic Maya traders on the western frontiers of the Maya lowlands.

Second, the continuing occupation of rural lands outside Copan fits in well with various new data indicating that the demographic situation in the southern lowlands by AD 900 and after was much more complex and varied than previously thought. Some large centres, particularly in northern Belize, suffered no disruption at all; some showed major population loss; some, like Copan, were abandoned but had a continuing rural population; and others were totally abandoned.

As we begin to obtain a clearer picture of the demographic pattern throughout the lowlands during and immediately after the ninth century AD, it will soon be possible to examine the relationships between this pattern and other factors such as soil degradation and access to water, raw materials and crops like cotton and cacao. Then, a new, testable explanation of the great changes in the Maya world between AD 800–1000 should be possible. ☐

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Graham Goddard (1938–1987)

PROFESSOR Graham Goddard, who made important contributions to the study of synaptic plasticity in the mammalian brain, died in a hiking accident in New Zealand on 15 January 1987.

Born in England in 1938, he spent most of his career in Canada before moving in 1981 to the University of Otago as Chairman of the Department of Psychology. After graduate work at McGill, he taught at the University of Waterloo and it was there, while investigating the role of the amygdala in avoidance learning in rats, that he made the discovery which established his reputation: in several regions of the brain low-intensity stimulation repeated daily eventually leads to the development of epileptic seizures. Kindling, as Goddard called this apparently permanent increase in excitability, continues to be widely studied as a stimulus-induced model of epilepsy.

More recently, first at Dalhousie University and then in New Zealand, Goddard turned his attention to long-term potentiation, a form of synaptic plasticity found in the hippocampus which provides a plausible model for information storage in the brain. His work in this field led to a series of discoveries of the first importance, including the demonstration of associativity, inhibitory suppression and heterosynaptic depression.

Graham Goddard brought exceptional analytical powers to the study of brain mechanisms, together with a psychologist's awareness of the importance of locating these mechanisms in the context of behaviour. His untimely death has deprived neuroscientists of an original and distinctive voice.

P.O. Andersen & T.V.P Bliss

Chemical oceanography

Global pollution measured by lead in mid-ocean sediments

Clair Patterson

ON page 278 of this issue[1], Veron and colleagues at CNRS and at Bordeaux University report their studies of the difficult, complex matter of lead pollution in the uppermost layers of outer deep-water sediments in the North Atlantic Ocean. It is common knowledge that heavy-metal pollution prevails within near-shore ocean sediments, and it might therefore be thought that either positive or conclusive negative evidence for lead pollution in the open oceans should also be readily found in pelagic sediments. But this is not the case: the processes that yield the pollution effects easily observable in coastal sediments also limit these effects from extending into the open oceans. Furthermore, mass-sedimentation rates in deep waters far from shore are so slight that there are serious difficulties in studying the very thin, uppermost, decades-old sediment layers involved, although pollution effects are enhanced by these small sedimentation rates. Such problems made investigators in the past reluctant to search for evidence of lead pollution effects in deep-ocean sediments.

Two hundred years ago, lead in the oceans did come from rivers, carried outwards by water transport, but the concentration in deep open ocean waters was then so small that it corresponds to those that now exist in purified laboratory waters (about 10^{-13} g Pb per g water). Today, much more lead contamination of the oceans comes from the atmosphere than from rivers. In earlier times the bulk of natural lead entering the oceans was weathered from continents and was included in silicate and humic particles that were deposited in relatively shallow shelf sediments. The small, aqueous, chelated remainder of river lead was rapidly sorbed on surfaces of phytoplankton which were then eaten by zooplankton in biologically productive off-shore shelf waters. This biologically processed lead was then deposited in local sediments with zooplankton fecal pellets and phytoplankton debris. The small fraction of soluble lead that escaped these efficient removal mechanisms was advected outwards by water currents to the open oceans, where it was removed from the upper, biologically active, thin, mixed zone by plankton, and finally sedimented in fecal pellets. Some of this lead was released from disintegrating pellets before they settled to the sediment layer, and formed a lower-water reservoir of soluble lead which was recycled upwards to the mixed layer, together with nutrients, to be removed and sedimented downwards again.

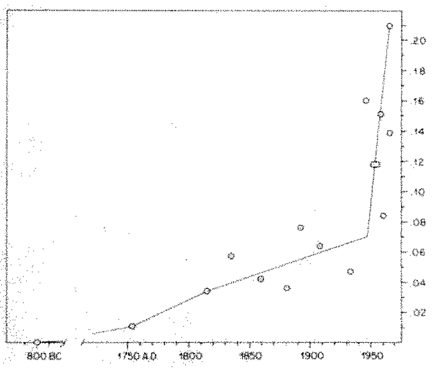

Fig. 1 Increase of industrial lead pollution in Greenland snow with time since 800 BC. Ordinate, concentration of lead (parts per 10^9) per kg snow; abscissa, age of samples (from refs 2,5).

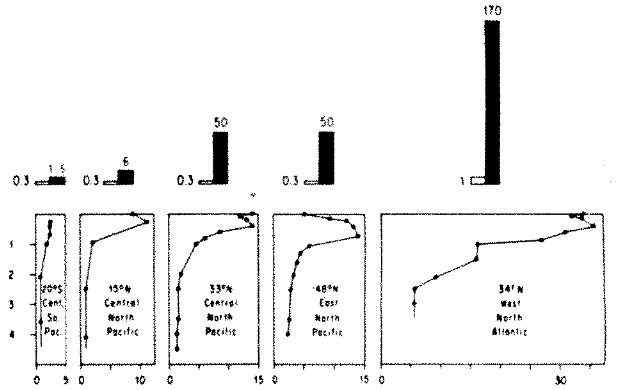

Fig. 2 Comparison of eolian (wind-borne) lead inputs with concentration profiles in the open oceans. Histograms show the net atmospheric lead input fluxes (10^{-9} g cm^{-2} yr), corrected for recycled sea spray. Filled columns, present day; white columns, ancient. Graphs show the lead concentrations of sea water (abscissa; 10^{-9} g kg^{-1}) per km depth (ordinate) (from refs 3,6,7).

Most chemical oceanographers believe that undisturbed natural pre-industrial shapes of depth–concentration profiles of lead in the deep open oceans can no longer be used for direct measurement because of contamination by industrial lead. It is uncertain, for example, whether lead concentrations in the upper 1-km thermocline zones of natural profiles are larger, nearly the same or smaller than concentrations in the underlying 4–5-km-deep water zones.

The timing and magnitudes of changes in lead concentrations in the Earth's atmosphere during recent centuries have been recorded in polar ice strata and were discussed[2] in a News and Views article last year (Fig. 1). These data indicate that direct inputs of lead carried by rain to open oceans increased enormously in the Northern Hemisphere during the past 50 years. These greater flows of lead from direct atmospheric input routes bypass coastal-sediment trap barriers, which allow only small flows of lead to the open oceans. Today, all measured lead concentration profiles show varying sizes of positive bulges in their upper 1-km-thick thermocline zones (Fig. 2). The bulges of greatest magnitude are located in the North Atlantic, where atmospheric inputs of lead to the oceans from rain are largest; intermediate bulges are found in the North Pacific, where atmospheric inputs are intermediate; and the smallest bulges are in the South Pacific, where atmospheric inputs are small. This geographical variation in pollution effects results from hemispheric differences among inputs of industrial lead to meridinal tropospheric circulation cells. Most industrial atmospheric lead is emitted to the northern westerly winds from North America, Europe and Japan, influenced by a latitudinal west-to-east decline of atmospheric inputs of lead to the oceans caused by short atmospheric residence times of lead-rich anthropogenic aerosols circling the Earth.

Temporal changes in lead concentrations in oceans can be recorded in coral growth layers. Shen and Boyle have recently made the first measurements[3] of these changes (dashed curve in Fig. 3) which define the increase of lead/calcium ratios in mid-North Atlantic coral layers during the past century. The congruence of this curve with that in Fig. 1 proves the existence of a close relationship between changes of lead concentrations in the troposphere and in ocean surface-layer waters, and it validates the existing interpretative model for ocean lead depth–concentration profiles. Industrial atmospheric lead emissions increased sharply after 1940 because of the large increase in production and burning of lead alkyls in automobile fuel. Emissions from North America were the source of most of the lead in mid-North Atlantic air before 1970, but the decline in the coral lead curve after that date is probably associated with the decline in alkyl lead emissions resulting from US government restrictions on sales.

Shen and Boyle also measured[4] stable lead isotope tracers in coral layers, and these data further substantiate the validity of the atmospheric pollution model by showing a close temporal coupling between changes in the isotope compositions of atmospheric industrial leads and oceanic surface water leads (solid curve in Fig. 3). Before 1880 seawater lead was natural, and possessed a high $^{206}\mathrm{Pb}/^{207}\mathrm{Pb}$ ratio characteristic of fluvial leads weathered from ancient shield drainages of North America. After this year, industrial leads, characterized by low $^{206}\mathrm{Pb}/^{207}\mathrm{Pb}$ ratios in leads of old ore bodies, began to be introduced to the Atlantic from smelter fumes in increasing amounts which, during the next 50 years, gradually reduced $^{206}\mathrm{Pb}/^{207}\mathrm{Pb}$ ratios in successive mixtures of natural and industrial lead in the North Atlantic mixed-surface zone (the residence time of lead in that thin layer is about a year), shown by the decline in the solid curve in Fig. 3 between 1880 and 1940. After 1960 this ratio in North American atmospheric industrial lead increased as a result of a higher proportion of anomalously radiogenic lead mined in Missouri, which was mixed with lead mined elsewhere from much older ore bodies, and used in the production of lead alkyls in the United States. This is reflected by a rise in the solid curve of Fig. 3 between 1960 and 1970. The decline in this curve after 1970 is probably associated with the increased importance of less radiogenic industrial lead emissions from Europe (added to the North Atlantic from easterly winds which then eddy northwards into the westerlies) as the proportion of lead emissions from the United States declined in the mixture of atmospheric leads over the North Atlantic.

It was in this context that Veron *et al.* carried out the measurements of industrial lead in open ocean sediments that they report in this issue[1]. Their data show an excess of lead in the uppermost 400 years of sediment layers in a pelagic core and the uppermost 200 years of layers in a deep outer shelf core (mixing by organisms during the past century extended to these depths) which cannot be accounted for by natural sedimentation processes, whose lead deposition fluxes were determined in contiguous deeper sediment layers. The magnitude of this excess lead equals about half the total integrated input flux of atmospheric industrial lead to the North Atlantic ocean surface, calculated from input fluxes measured at specific dates normalized to the slope of the Greenland ice lead curve before 1940, and normalized to the lead alkyl consumption curve for the United States, Canada and Europe after 1940. The magnitude of excess deep-ocean sediment lead determined by Veron *et al.* is also approximately equal to the total amount of industrial lead in the North Atlantic thermocline 'bulge', suggesting that about half the industrial lead input is sedimented and the other half resides in the water column. Simplifications involved in these interpretations may have introduced errors but, overall, the findings reported by Veron *et al.* are consistent with, and develop and extend, the prevailing model that explains the concentration of lead in the oceans. ☐

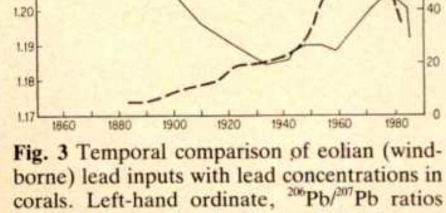

Fig. 3 Temporal comparison of eolian (wind-borne) lead inputs with lead concentrations in corals. Left-hand ordinate, $^{206}\mathrm{Pb}/^{207}\mathrm{Pb}$ ratios (solid line); right-hand ordinate, Pb/Ca in nmol mol^{-1} (dashed line); abscissa, year (from ref. 4).

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Conservation biology

Problems in leaving the ark

Anna Marie Lyles and Robert M. May

UPSTAIRS in the Brooklyn Museum is a beautiful example of a favourite victorian plaything: a winding line of wooden animals wait in pairs, queueing up to enter Noah's ark. The ark is increasingly being used as a metaphor for conservation programmes, usually based in zoos or aquaria, that aim to preserve species whose continued existence in the wild is threatened by the rising tide of humanity. Contemporary zoos face great problems in embarking their animals, with a foreseeable need to keep 2,000 or more species of terrestrial vertebrates (not to mention other endangered animals) afloat indefinitely[1]. This is beyond the capacity of existing institutions, and already poses difficult problems of choice that will get worse as time goes by[2].

The hope, of course, is that someday, in a better-ordered and less human-centred world, it will be possible to return animals to flourish in something close to their original habitats. In this sense, the concept of the zoo as an ark represents a change from its traditional role as essentially a 'cabinet of curiosities', a museum of living things, with traffic being one way: in and not out. For a few species, someday is now, with deliberate efforts to reintroduce captive-bred animals into their indigenous habitats. In some cases, such reintroductions have followed extinction of wild populations, whereas in others reintroductions have been made to enhance genetic variability or to bolster a declining population. These experiences have led to a growing appreciation of the genetic, ecological and behavioural problems that species may encounter on leaving the ark (ref. 3; M.R. Stanley-Price, personal communication).

One lesson is that the conditions originally causing the wild population to decline should be identified, and if possible remedied, before attempts at reintroduction are made. The story of the endemic Hawaiian goose, *Branta sandvicensis*, illustrates this point[2]. The birds live in rather inaccessible places, and the causes of their decline to near extinction in the middle of this century have not been adequately identified, much less corrected. About 1,761 captive-reared birds were released between 1960 and 1978, but in 1978 the wild population of about 750 birds — although no longer on the brink of extinction — still was not self-sustaining.

A second lesson is that it is important to choose an appropriate release site. Smaller animals with fairly precise habitat requirements or complex life-cycles may require more exhaustive feasibility studies than do larger generalists. The choice of an appropriate release site can become even more difficult when the species is extinct in the wild. Assessment of its ecological requirements will depend upon inferences drawn from closely related

Eric Hoskins

Hawaiian goose Branta sandvicensis.

species, research on captive animals and observations made during pilot-release programmes. The initially unsuccessful programme[5] to reintroduce the cheer pheasant, *Catreas wallichi*, into Pakistan (where they were thought to be extinct) exemplifies the importance of follow-up programmes. Surplus eggs from British breeders were shipped to Pakistan, hatched and released. Subsequent survival was very low. Radiotelemetry was therefore used to determine the habitat requirements of the birds, which led to the release site being changed and consequently to higher survival.

A third lesson involves a cluster of concepts related to the idea of reintroducing groups of animals whose overall genetic makeup will enhance their success. There seems a good *a priori* reason to suppose that stocks containing intact natural genetic variation will do better[6]. Unfortunately, this variation within the stocks will seldom be available, because captive propagation and reintroduction plans are all too often last-ditch efforts to stave off extinction, and founders are collected after substantial genetic variation has already been lost. Some currently publicized examples, such as the California condor or the black-footed ferret, bring this home.

Given that we rarely have the luxury of reintroducing the full genetic variation found in the original indigenous stocks, there are several ways of selecting compromises that may approximate the correct locally adapted genotype. One idea is to maximize genetic heterogeneity in the released animals by hybrid matings among populations or subspecies. The hope here is that, after release, natural selection will produce the correct 'locally adapted' genotype. This approach has, for example, been adopted for the reintroduction of swift foxes, *Vulpes vulpes*, to the Canadian prairie and for collared lizards in the Missouri Ozarks of the United States[7,8]. But this hybridization approach can result in disaster. An example is the hybrid ibex, *Capra ibex*, which was reintroduced into Czechoslovakia, and which proceeded to breed in the wrong season[9].

A different idea is to try to obtain stock for reintroduction from the subspecies or population most likely to have local adaptions, even if this means relatively low genetic variability. Such animals could derive from indigenous stocks (taken into captivity, bred up and reintroduced), or from near neighbours, often a population in a similar habitat. Because animals from different populations are often mixed up in captive collections, this approach is not always possible, but has, for example, been used for reintroduction of the fish *Pociliopsis occidentalis* in Arizona[10].

Even if a captive population starts with a sufficient number of founders to keep intact most of the natural gene pool, and even if these are kept in naturalistic enclosures where conspicuously unusual selective forces are avoided, loss of the wild genetic variation (that is, domestication) is inevitable if the animals are kept long enough[11]. It is thus desirable to keep the number of generations between capture from the wild and reintroduction as low as possible. These ideas are illustrated by efforts to preserve the Lord Howe Island wood rail, *Tricholimnas sylvestri*[12]. This species declined under the depredations of introducted feral animals, with fewer than 20 birds remaining in 1980. Five pairs were captured and a breeding programme was established on the island. At the end of 1983, 74 birds were reintroduced; the population was back to 200 or more birds in 1984.

Eric and David Hoskins

Looking to a bright future? The otter Lutra lutra.

A fourth lesson is that behavioural patterns are an important consideration in reintroduction programmes. Social animals should be released as cohesive groups whenever possible, to minimize stress and to facilitate reproduction. Gradual acclimitization of individuals to each other and to the new environment seems the best way to present the groups from scattering on release. This is often achieved by shipping the animals to the release site and keeping them in pens where social groups are assembled. The animals can be fed and provided with shelter for some time after the gates are opened, both to ease the transition for the animals while they learn to use wild resources and also to make monitoring easier.

Such techniques smoothed the reintroduction of the Arabian oryx, *Oryx leucoryx*[13]. Direct persecution by hunting exterminated this animal from the wild in 1972. Fortunately, captive herds had been established during the 1960s and reintroductions into the Oman desert began in 1980, using behavioural data from a closely related species. Seventeen animals were shipped to the Oman and penned as two herds. When social patterns seemed stable and the full range of sexual behaviours was seen, the release was begun. This reintroduction seems to be a success, with 15 live births and only 6 deaths between 1980 and 1984. The project has also gained local support by employing local bedouins as wildlife rangers and drivers.

Reintroductions of territorial animals may be more difficult, as each group may have to be released at a different site. In England, the otter *Lutra lutra* has recently been reintroduced[14] into patches of its native range from which it was lost in the 1950s and 1960s. Pollution by organochlorine insecticides, coupled with habitat loss and disturbance, contributed to the sudden decline and fragmentation of populations of these otters. Four breeding units (2 females and 1 male in each) were assembled from young animals bred in captivity, acclimatized on-site and released at different sites between 1983 and 1985. These groups seem to have expanded their home ranges successfully and have begun to breed.

The behavioural similarity of released and wild otters suggests that much otter behaviour is innate. This suggests a fifth lesson, namely that animals with flexible behaviour patterns may be very difficult to reintroduce. By analogy to the process of (relatively slow) loss of genetic variability by domestication, culturally determined repertoires of behaviour may be irretrievably lost in zoos. Captive-born animals may be 'rehabilitated' through elaborate training, but there are limits to the skills that can be taught; captive-bred individuals may have limitations to their learning abilities that significantly reduce their viability when they are released.

Victorian Noah's arks and present-day efforts to preserve animal species both focus mainly on mammals and birds, as has this article. We hope to see more concern for endangered species of arthropods and other non-chordate phyla. Insofar as most of these creatures tend to have behavioural patterns that are more 'hard-wired' than those of higher vertebrates, to have higher intrinsic capacities for population growth, and to have relatively small physical sizes (making it easier to keep relatively large populations), their chances of leaving the ark successfully may be higher than for many vertebrates. On the other side of this coin are the very specialized habitat requirements of many of these arthropod and other species. ☐

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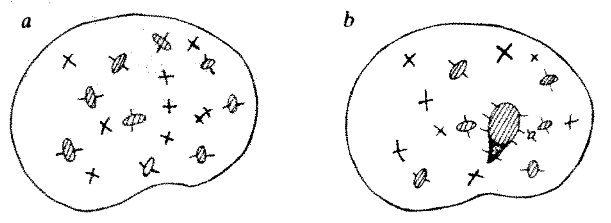
Protein structure

New light on old defects

Roger H. Pain

MYOGLOBIN, the 'hydrogen atom of biology', shares with other globular proteins the characteristic that its 1,261 non-hydrogen atoms are remarkably well packed (although not as closely as a box of tennis balls). As I have discussed previously in a News and Views article[1], this relaxed packing is associated with compulsive motion of the atoms in a manner which, like the chemical and physical nature of the protein interior, is anisotropic and which is believed to contribute to the biological function of these molecules. Two quite different experimental approaches, solution compressibility and X-ray crystallography, have now been used by Gekko and Hasegawa[2] and by Frauenfelder *et al.*[3] to shed more light on these basic attributes of protein structure.



A rough two-dimensional projection of channel production by rearrangement of the free-volume increments associated with mobile defects. The channel is shown in *b*. Crosses, regions of good binding which are potential defects; shaded ellipsoids, defects showing broken hydrogen bonds (re-drawn from ref.4).

There are three kinds of free space within a protein structure: the inevitable vacuum left if and when the atoms are packed ideally to the limit of their van der Waals' radii; spaces of greater than atomic size frequently occupied by water molecules and termed cavities; and defects where atoms and groups are on average separated by subatomic distances. Such defects, or regions of poor bonding interconnected by protein fabric, can provide a high cooperative mobility of free volume and its associated potential energy. These have been called 'mobile defects'[4] and can provide pathways for the penetration of small molecules to the interior of the folded protein (see figure). The question arises as to whether the defects are the result of the forces that lead to condensation of the polypeptide chain being opposed by thermal agitation, or whether they represent the optimal packing of each particular chain.

Gekko and Hasegawa[2], using measurements of sound velocity, extend existing data on compressibility to 25 proteins, including myoglobin. All the globular proteins so far studied (with the exception of subtilisin) show a positive adiabatic compressibility β_s, suggesting that the cavities and/or defects *can* be reduced in size, although quantitation is subject to the uncertainty of the negative contribution of hydration to β_s. This finding is strengthened by the demonstration that values of β_s correlate reasonably well with the independently measured volume occupied in solution by unit mass of protein, V_0. In other words, the larger the volume occupied by a given length of folded polypeptide chain, the more the molecule can be compressed.

Because bulk properties must have their origin in the detailed molecular structure it was hoped that it might be possible to identify important features by correlating compressibility with various features of the 25 different proteins. Proteins with a high content of helical structure seem to be more compressible than those with low helix content, although Gekko and Hasegawa knew that correlations could reflect other properties — in this case multi-subunit and multi-domain structure. Their result could, however, mean that the rather densely packed helical structures are prevented, perhaps by their own rigidity, from packing together in a way that minimizes the defect space. The acid test should be a careful study of the X-ray structures of helical and non-helical proteins.

The correlation of compressibility with the paraffin-like nature or hydrophobicity of the component amino-acid residues reveals a tantalizing result that is not easy to translate into detailed structural terms. Overall hydrophobicity of the protein correlates positively with compressibility whereas, when the content of individual residues is examined, correlation of β_s is observed with only two out of the six major hydrophobic residues. Clearly, some other, unknown property of these residues, possibly associated with their 'packability', is involved.

It is possible to draw conclusions about the dynamic fluctuations of a protein using statistical thermodynamics as originally discussed by Cooper[5]. Gekko and Hasegawa show that the extent of the volume fluctuation is on average 0.3 per cent of the total volume in many proteins. As they point out, this is more than enough, if the increased volume is localized at a few points within the folded protein, to accommodate extraneous molecules in the manner proposed in the mobile defect hypothesis.

Frauenfelder and co-workers[3] approach the question of the basis of defect and cavity space in a different way by examining in detail the atomic coordinates of myoglobin obtained from X-ray crystallographic analysis at 300 and at 80 K. They observe a general shrinkage of the protein crystal for this reduction in temperature, expressed as a 5 per cent reduction in volume of the crystallographic unit cell and a 3 per cent decrease in that of the protein molecule. The fact that cooling was achieved without use of a cryosolvent suggests that the shrinkage is associated primarily with the decrease in thermal energy. Taken together with the compressibility studies, this suggests that a proportion at least of the free space in a protein structure is the result of thermal 'expansion' energy and not just limitation of folding.

Perhaps the most interesting aspect of the work of Frauenfelder *et al.* is the finding that the shrinkage has little effect on the dimensions of the helical cylinders and, similarly, that the proportional shrinkage of the larger cavities is small. In consequence the largest volume change must be occurring in the small packing defects. It is intriguing to postulate, therefore, that it is these defects that are most susceptible to deformation by thermal energy, and by extension that they, more than other regions, are subject to thermal fluctuations in the protein interior. This could lead on a longer timescale to mobility of the associated potential energy to create transitory larger defects in specific locations into which an extraneous molecule such as oxygen, water or acrylamide could fit[1]. Subsequent redistribution of the energy would allow the molecule to move through the protein as easily as a fishmonger through a debutantes' garden party, as proposed[4] in the mobile defect hypothesis.

Boyles' law has been redefined as "the greater the external pressure the greater the volume of hot air". The fact that the formidable amount of work involved in the new studies[2,3] relating temperature, pressure and volume has not led to instant understanding of protein dynamics shows how unready the protein molecule is to reveal its secrets. But the results do promise fresh insights from the combination of thermodynamic and structural experimentation. ☐

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Mathematics

Hilbert's sixteenth problem

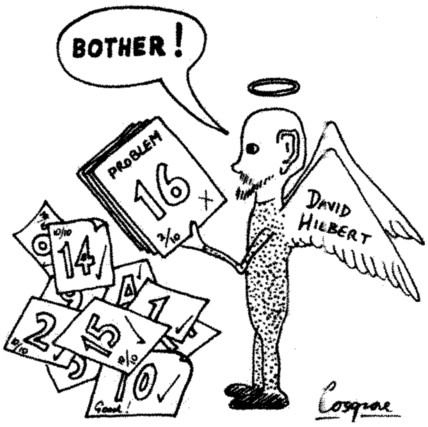
Ian Stewart

IN 1900 David Hilbert delivered a celebrated lecture before the International Congress of Mathematicians in Paris. "The close of a great epoch," he said, "not only invites us to look back into the past but also directs our thoughts to the unknown future." Hilbert emphasized the importance of specific problems in mathematical research, and listed 23 problems which seemed to him to be of fundamental interest. It is a measure of the maturity of today's mathematics that most of Hilbert's problems now have answers, but some remain stubbornly intractable, the most notorious being the Riemann hypothesis. The sixteenth of Hilbert's problems comes in two similar but largely unrelated parts: the first asks how the different branches of a plane curve can be arranged; and the second raises the analogous problem of the number of limit cycles arising from a differential equation in the plane. There are some important recent developments, not all positive, on this second part of Hilbert's sixteenth problem.

Before describing the current state of play, I will look at the origins of the problem in more detail. In the late nineteenth century, mathematicians were starting to take a serious interest in nonlinear differential equations. In particular they worked on differential equations of the form $dy/dx=Y/X$, where X and Y are polynomials in x and y. In general it is not possible to write down explicit solutions to such equations, but Henri Poincaré discovered that a great deal of information can be obtained by topological means. Rewrite the equation as a system $dx/dt=X$, $dy/dt=Y$, where t is a new variable, thought of as representing time. As t varies, the solution $(x(t),y(t))$ describes a curve in the plane. This curve depends on the initial conditions $(x(0),y(0))$, and by considering all possible initial conditions one obtains a system of curves called the phase portrait of the equation. The phase portrait gives a geometrical picture of all possible solutions to the differential equation, and is a powerful tool for a qualitative study.

In a fundamental re-direction of research on differential equations, Poincaré determined the basic topological properties of such phase portraits. In particular he recognized the importance of limit cycles, which occur when a curve in the phase portrait closes up on itself forming a loop. A limit cycle corresponds to a periodic solution of the differential equation, in which the system repeats the same behaviour over and over again, and hence has genuine physical interest. (In practice, some extra technical requirements are imposed when defining limit cycles, but I will omit these here.)

The second part of Hilbert's sixteenth problem asks how many such limit cycles can occur, for polynomials X and Y of given degree. When Hilbert raised the question, it was not even known whether the number of limit cycles must be finite. This weaker version of Hilbert's problem appeared to be solved affirmatively when H. Dulac wrote a lengthy memoir (*Bulletin de la Société Mathématique de France* **51**, 45–188; 1923). The crucial step in Dulac's proof is to show that an infinite number of limit cycles cannot accumulate around a single point of the phase portrait.



Some time later, Yu.S.Il'yashenko of Moscow State University (*Uspekhi Mat. Nauk.* **37**, 127; 1982) noticed an error in one of the lemmas used by Dulac to establish his main theorem. Now, Il'yashenko has managed to repair some of the results in Dulac's memoir and to prove the finiteness of the number of limit cycles under more stringent conditions. In particular he shows that for polynomials X, Y of degree two, the number of limit cycles occurring in any finite portion of the plane is finite; and that for "almost all" polynomials of degree two, the number of limit cycles in the whole plane is finite. The proofs use modern developments in singularity theory and the theory of normal forms.

A proof of finiteness of the number of limit cycles for polynomials of degree higher than two would be a major step towards solving Hilbert's sixteenth problem. But Hilbert asks for more: namely, the exact number. To be precise, let $H(n)$ be the maximum number of limit cycles that can occur when X and Y have degree n. The known results, described above, cannot even show that $H(n)$ is finite. However, in all known examples $H(n)$ is not just finite, but fairly small. The gap between what is conjectured that exists and what is known is enormous.

N.N. Bautin (*Mat. Sbornik* **30**, 181–196; 1952) found a pair of polynomials X and Y of degree two, for which three limit cycles occur. Thus $H(2)$ is at least 3. This result was bettered by Shi Sonling (*Scient. Sinica* **23**, 153–158; 1980) who found an example with four limit cycles. Thus $H(2)$ is at least 4. This example is a small (and very complicated) perturbation of the system given by $X=-y-10x^2+5xy+y^2$, $Y=x+x^2-25xy$.

The best results to date are that $H(n)$ is at least $\frac{1}{2}(n^2+5n-14)$ for even $n \geq 4$, at least $\frac{1}{2}(n^2+5n-26)$ for odd $n \geq 9$, and that $H(3) \geq 5$, $H(5) \geq 14$, $H(7) \geq 27$. The first two results here are due to N.F. Otrokov (*Mat. Sbornik* **34**, 127–144; 1954). That for $H(3)$ is due to K.S. Sibirskii (*Different. Uravneniya* **1**, 53–66; 1965) and Shi Sonling (*Acta Math. Sinica* **4**, 300–304; 1975); the last two follow from a general result of Il'yashenko (*Mat. Sbornik* **78**, 360–373; 1969).

Nonlinear differential equations are important in all sciences and are at the forefront of some of the most active areas of current mathematical research. Today's methods for analysing nonlinear differential equations give an excellent understanding of many general phenomena. Moreover, numerical methods, implemented on a computer, can provide very detailed information on any specific differential equation of interest. Hilbert's sixteenth problem falls neatly in between. It concerns a class of equations (those defined by polynomials X and Y of degree n) which is too restricted to be amenable to general methods, yet too broad (having too many free parameters) to yield to numerical analysis. Additionally, Hilbert's problem asks for global information on the entire phase portrait, whereas many of the most powerful general results are only local.

Mathematicians tend to think of differential equations in two variables as being trivial. This is perhaps true of specific equations, or of the local behaviour of arbitrary equations. The status of Hilbert's sixteenth problem illustrates that the global behaviour of multi-parameter families of equations continues to pose almost insuperable problems. We can sympathize with Hilbert's opening words of 1900: "Who of us would not be glad to lift the veil behind which the future lies hidden; to cast a glance at the next advances of our science and at the secrets of its development during future centuries? What particular goals will there be toward which the leading mathematical spirits of coming generations will strive? What new methods and new facts in the wide and rich field of mathematical thought will the new centuries disclose?" □

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Immunization against AIDS in humans

SIR—The discovery of a diversity of subtypes of human immunodeficiency virus (HIV)[1-3] immediately raised the possibility of major difficulties in the development of a vaccine against AIDS (acquired immune deficiency syndrome) because a humoral response induced by one HIV strain may generate neutralizing antibodies only against that strain (that is, a type-specific response) and not against other HIV strains. In fact, this is precisely the case in most reported animal experiments[4]. This difficulty prompted us to investigate whether a cell-mediated immune response, based on a signal recognition system that activates both cytotoxic T cells and T-cell-dependent antibody-secreting B-cell clones, might overcome the limitation of type-specific responses to potential immunogens. We wish to report the results of primary immunization of one of us (D.Z.) by one of two vaccines that we believe will stimulate a cell-mediated immune response against HIV infection.

We have previously shown that, after immune activation, cells infected with HIV release virus[5]. Before doing so, they proceed through an immunogenic stage where viral antigens are present on the cell membrane (unpublished results). Activation of a cell-mediated immune response should therefore hinder viral dissemination both by destroying these cells (by means of specific killer T cells) and by the (antibody-mediated) neutralization of free virus. To induce such a response in HIV seronegative immunologically normal individuals, we have used a recombinant HIV–vaccinia virus which has the advantages of triggering a strong cell-mediated immune response and not producing recurrent infection. The recombinant virus[6,7] contains the *env* gene of a clone of the HTLV-III_B strain[8] of HIV. The envelope sequence encodes the gp160 protein which includes both the outer gp120 and the transmembrane gp41 peptides. Cells infected with the recombinant virus express gp160 on their membranes[7].

Preliminary experiments in several species of animals by others[7,9] and in *Cercopithecus*, baboons and one chimpanzee by us in collaboration with B. Moss, M. Robert-Guroff and R.C. Gallo[10] have demonstrated the safety of the recombinant virus. In addition, our work on *Cercopithecus* has shown the absence of measurable immune impairment during and after immunization.

Table 1 HTLV-III-induced proliferative response and IL-2 receptor expression following immunization

Stimulator		Proliferative index		% IL-2 receptor positive cells	
		D.Z.	NNII	D.Z.	NNII
	Dilution				
HTLV-III_B	1/50	7	<2	17	<3
	1/100	20	<2	–	<3
	1/200	26	<2	14	<3
	1/400	12	<2	12	<3
	no virus	1	1	<3	<3
UV-irradiated HTLV-III_B	1/20	3	<2	8	<3
	1/50	8	<2	10	<3
	1/200	5.3	<2	5	<3
	1/400	5.8	<2	–	<3
	no virus	1	1	<3	<3

Fresh peripheral blood lymphocytes from D.Z. (63 days after immunization) and from a normal non-immunized individual (NNII) were isolated by Ficoll-Hypaque and suspended in RPMI supplemented with 10% heat-inactivated normal human serum. To aliquots of 10^5 cells in a final volume of 0.1 ml of medium, were added 0.1 ml of medium containing dilutions of UV-inactivated (2,500 joules) HTLV-III_B or HTLV-III_{RF} derived from a stock solution of live virus (1 μg ml^{-1} centrifuged from supernatants of infected H9 cells provided by R.C. Gallo). Cells were cultured at 37 °C in a 5% CO_2 incubator. To measure proliferative index, six days after stimulation the contents of each well were labelled with 1 μCi ^3H-thymidine for 8 h, after which cell ^3H was measured. The mean results of triplicate samples divided by the control value (no virus) are shown. A monoclonal antibody against the Tac antigen (provided by T. Waldman) and a goat antimurine antibody coupled to fluoresceine were used to determine the percentage of IL-2 receptor positive cells. The mean results of duplicates are shown.

Primary immunization of D.Z., who is HIV seronegative, involved scarification with 10^7 plaque forming units of the recombinant virus. Following immunization no systematic reactions (temperature, chills, headache or stiff neck) or regional reactions (enlargement of draining lymph nodes or lymphangitis) were observed. A 1.5 cm pustule appeared at the scarification site by day seven and followed the usual evolution of a good response to vaccinia. The immune system was monitored weekly over a nine-week period by measuring CD4 and CD8 cell numbers as well as the *in vitro* production of interleukin-2 (IL-2) from the cells. No immune defect was detected by either assay. The immune response to vaccinia was measured by enzyme-linked immunosorbent assay (ELISA) and showed a 1:2430 titre 30 days after immunization. Antibodies against HTLV-III_B antigens were also detected. On day 30 D.Z.'s serum was highly positive in a competitive inhibitor test for antibodies against gp41. On days 30 and 63 we detected neutralizing antibody[12] against HTLV-III_B with a titre of 1:40 but not against HTLV-III_{RF}, a subtype also referred to as HAT, which diverges from the B strain by over 1,000 nucleotides[13].

Cellular immune response was studied 63 days after immunization. Fresh peripheral blood lymphocytes stimulated either by UV-irradiated HTLV-III_B or HTLV-III_{RF} were used as responders. Stimulation with HTLV-III_B produced a high level of response as measured by the proliferative index as well as by the number of IL-2 receptor positive cells (see table). HTLV-III_{RF} stimulation also activated the cells but to a lesser extent. A similar degree of activation of the responder cells was observed when fixed HIV-infected autologous cells were used as stimulators.

These results show that immunization of humans with a recombinant vaccinia virus expressing HTLV-III_B envelope glycoproteins can trigger a primary immune response not only against the immunizing strain but also, albeit to a lesser degree, against a very different strain. Preliminary confirmation of this result has come from immunization of a small group of Zairians, all of whom were HIV- seronegative volunteers and immunologically normal as assessed both by CD4 and CD8 positive cells and by IL-2 production. Two of these individuals developed a significant titre of neutralizing antibody against the HTLV-III_{RF} strain.

To obtain an amplification of the primary immune response, we have recently boosted D.Z. and a few other seronegative volunteers who had received the recombinant virus vaccine. If this boost is not sufficient to produce a high immune response associated with neutralizing antibodies against different HIV strains, alternative approaches — such as the use of multiple recombinant viruses, each expressing a different subtype of HIV gp160 or the use of alternative boosters — will be explored. We would wish to overcome any limitations imposed by subtype diversity before we could properly consider any large scale clinical trial of the vaccine.

While the recombinant HIV–vaccinia virus is our initial choice for immunizing seronegative individuals, for immune deficient AIDS patients we are attempting to induce a cell-mediated response by administration of non-infectious, fixed autologous cells expressing HIV antigens on their surface. We shall report full details of the protocol and the outcome of the first immunizations of that kind, which have been carried out in Zaire, in due course. All our work in Zaire has the full support of the Zairian Ethics Committee.

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Cell fusion in viral diseases

Sir—Lifson *et al.*[1] and Sodroski *et al.*[2] report that syncytia production by the human immunodeficiency virus (HIV) in susceptible cells is a characteristic feature of infection. They suggest that this type of cell fusion is associated with a differentiation antigen (CD4) and requires only the expression of the virus-specific glycoprotein of HIV. The fusion could contribute directly to the development of the specific cytopathology.

During a study of syncytia produced by the parainfluenza 3 viruses in malignant KB cells, we have observed that during the first replicative cycle up to 30 nuclei can fuse in a syncytium at the end of the eclipse period and before the release of mature virions[3,4]. Polykaryocytes are only generated at low multiplicity of infection; microcinematographic analysis indicates that they result from the extension of the fusion process from infected to normal cells[4].

In a more recent series of experiments using temperature-sensitive mutants (ts) of vesicular stomatitis virus (VSV) in rat transformed XC cells, we observed that pronounced polykaryocytosis is obtained at the nonpermissive temperature as long as the selected mutant is able to express the envelope-associated G antigen[5]. Such extensive polykaryocytosis can be generated from a single infected cell[6] because the glycoprotein propagates through intercellular communications from cell membrane to cell membrane in the absence of any viral replication. Occasionally, the primary infected cell fuses to the proximal side of the membrane of its closest neighbour, leaving the distal side temporarily free[6]. Our results have been confirmed by Florkiewicz and Rose[7], who demonstrated the fusing capacity of cloned G antigen integrated in L-cells.

When mice are inoculated intracerebrally with the ts VSV mutants, a spongiform encephalopathy can occur[8]. The lesions are disseminated in the grey matter, both in the brain and the spinal cord. The mutant virus multiples at a very low level in the brain and not at all in the spinal cord, and can be detected neither by infectivity assay nor by electron microscopy. The labelled dendritic processes are swollen, resulting in a characteristic vacuolization marked by peroxidase-labelled antibodies to the G antigen. They also show areas of abnormal configuration of the plasma membrane, with alternating dark and light lamina[8].

We postulate that the presence of a relatively small number of VSV-infected cells can create widespread havoc either by cell-to-cell extension of the G antigen or by its propagation along the neuronal processes to considerable distances. The cytopathogenicity of VSV and the mode of extension of the lesions it induces may have parallels in both slow virus diseases and AIDS (acquired immune deficiency syndrome).

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Polar ice test of the scale dependence of *G*

Sir—A broad class of quantum gravity theories predicts the existence of additional, finite-range, Yukawa-like interaction terms in the gravitational potential at the newtonian limit[1]. Because the departures from newtonian behaviour produced by these terms are small and thought to be significant at ranges of a few metres to a few kilometres, any deviation from the classical form of gravity will be difficult to detect in the laboratory, so that experiments performed on a geophysical scale are a useful test of modern unified theories. One way in which a departure from classical behaviour can be visualized is as an apparent dependence of the newtonian gravitational constant, G, on the distance separating the attracting masses. A difference of nearly one per cent between the value of G determined in the laboratory (accurate to slightly better than one part in 10^4)[2] and the value of G measured over distances of the order of one kilometre may have been detected by Stacey and his coworkers in analyses of variations in the acceleration of the Earth's gravity, g, in deep mines[3,4]. Their results are not conclusive because the density of the attracting mass (the rock layers surrounding the mines) is not adequately known and is certainly inhomogeneous, and the possibility of regional gravity anomalies cannot be eliminated. Measurements of g in profiles through the ocean have also been suggested[5]. Although the density of seawater varies because of internal waves and small-scale mixing processes, it is certainly more uniform than that of rock. A major limitation in an oceanic measurement of G would be due to instrument motion and inadequate knowledge of the gravity meter's location within the water column.

Here we propose an alternative experiment using profiles of g obtained in boreholes through the polar ice cap. This offers several advantages over previously suggested approaches. The density of pure ice is controlled by temperature once the hydrostatic pressure is adequate to close cracks and squeeze out air inclusions[6]. Polar ice is remarkably free of impurities[7], and we believe its density can be predicted from an equation of state with an accuracy approaching a few parts in 10^4 below depths of several hundred metres. This is likely to hold more strongly for the Antarctic than for the Greenland ice cap owing to the greater age of the ice and a reduced input of terriginous contaminants. The density of polar ice is relatively homogeneous due to lateral flow of the ice sheet and uniform internal pressures from loading. There are also no difficulties from random, local accelerations of a gravimeter suspended in a borehole on the ice cap. Therefore, a polar ice borehole determination of G will have the instrumental stability and simplicity of the mine experiments along with the favourable density characteristics of an oceanic G determination.

There are currently at least two deep holes in the polar ice that are suitable for the proposed experiment. The first of these is located in south Greenland at Dye 3, and penetrates 2,037 m of ice to bedrock in a region of rugged basement relief[8]. The second hole is at the Vostok Base in Antarctica and is over 2,000 m deep in an ice sheet of about 3,700 m thickness[9]. Both holes are filled with a hydrocarbon fluid having a density similar to that of ice to ensure that they do not close under hydrostatic pressure.

The experiment is envisaged as follows. A standard LaCoste–Romberg borehole gravity meter (LaCoste & Romberg Co., Austin, Texas) is lowered into the hole and a series of mesurements of g at precisely determined depths is obtained. As this type of instrument measures g only in a relative sense and is subject to long-term drift, it must be calibrated elsewhere against an accurate absolute gravity meter[10]. Location in the hole can be determined by measuring the length of an invar wire under constant tension stretching from the instrument package to the surface. It can also be inferred from measurements of the ambient pressure in the borehole provided the density of the infilling liquid as a function of depth is known. More complex methods of depth measurement that could be used include surface radar tracking of a reflector on the borehole package or marine acoustic navigation techniques using a set of acoustic transponders on the ice interrogated in turn by one on the instrument package. In any case, an accuracy of one part in ten thousand should not be difficult to achieve. In practice, it will also be necessary to correct for gradients in gravity caused by topography at the ice–rock interface and variations in the thickness of the ice sheet. The form of such topography can be measured using precisely navigated ice-surface radar, a method in standard use by glaciologists[11]. The major remaining source of systematic error is the presence of regional gravity anomalies caused by anomalous masses in the crust and mantle underlying the ice. This argues strongly for performing the experiment in more than one borehole at different sites.

An experiment done over a 1 km vertical interval in which the uncertainties in ice density, gravity and depth within the borehole are respectively 1 part in 10^4, 9μ Gal (9×10^{-8} m s^{-2}), and 4 cm, will yield a value for G with an accuracy of 1 part in 10^4. A tenfold degradation of this accuracy goal would still allow a meaningful comparison to be made with the value of G seen by others on this scale.

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Mendel's legacy remains untarnished

SIR—Van Valen[1] suggests that I share the widespread view that Mendel falsified some of his data[2]. In fact, I do not, though it is certainly the view of sources quoted by the author whose book I was reviewing[3]. I plead guilty to having tried to save space but not to iconoclasm. It is doubtful, however, if the accusations against the other "historic figures" (Burt and Dawson/Woodward) with whom I misleadingly coupled the name of Mendel would "also dissolve if similarly scrutinized".

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Are 'pragmatypes' acceptable?

SIR—The rules for the designation of type specimens regularize the procedure for establishing the acceptable names for species. They minimize possibilities for dispute following advances in taxonomic understanding. For example, when a 'species' is found to be two species, which had been confused with each other, then the segregate which includes the holotype retains the existing name. If no holotype was designated when a species was originally described, a sub-sequent taxonomist may designate a specimen from its type series as a lecto-type. When the type material has been lost a neotype may be designated.

A notable advance this century has been the recognition of sibling species complexes. In many cases, internal features are the primary characters by which such species have been recognized. Thus, cytotaxonomy has employed banding patterns in the giant chromosomes in dipteran salivary glands, and electro-phoresis has been used to highlight species-specific differences in enzymes. In such cases, the value of an old holotype specimen, impaled on a corroding nineteenth century pin, is minimal. Indeed, its state is likely to be an impediment to sensible decisions regarding the acceptable names for newly recognized species that had previously all carried the name attached to this holotype. This sort of experience caused Mattingly[1] to coin the term 'mommet' for such holotypes.

Recently, examination of sibling species–polymorphism complexes in Phoridae (scuttle flies) has led to recognition of the taxonomic value of features of the gut. These include an unusual crop mechanism[2] and the rectal papilla number, the external correlates of which have not always been discovered or in some cases exhibit an overlapping range. Thus most useful reference specimens are now the slide-mounts of dissected flies in which all the taxonomically familiar morphological features are displayed along with a display of the rectum.

Sorting specimens on rectal papilla number in the *Megaselia pulicaria* complex indicates that there are at least three species: A, B and C. The pinned holotype of *M. pulicaria* (Fallén) has been examined. Its rectal papilla number cannot be determined. What little of the external morphology can be inspected only allows an opinion that it probably belongs to species A.

It would seem sensible to designate a slide-mounted specimen of species A as a replacement type for *M. pulicaria*. This procedure would make Fallén's holotype of *M. pulicaria* a specimen of historical and curiosity value only. The primary reference specimen for the species, for nomenclatural purposes, therefore becomes the replacement type.

I should like to suggest the term 'pragmatype' for such replacement types. Clearly the designation of pragmatypes should only be countenanced when the primary characters used to separate newly recognized sibling species are features not observable in existing types. I intend to designate pragmatypes shortly, in a paper in preparation on the *M. pulicaria* complex, unless sound reasons are advanced against the proposal.

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Search for natural isotope superlattices

SIR—The search for the formation of superlattice-type structures has yielded a good harvest for condensed matter scientists. Period doublings, commensurate–incommensurate transitions, spontaneous ordering of voids in metals under neutron irradiation, and so forth, all represent examples of self-organization (order–disorder transitions) in non-equilibrium systems in the sense of Prigogine synergetics[1]. In all cases of self-ordering two requirements are important: first, there should be some interaction between ordering species distinct from the host interactions; second, the ordered state of the minority species (voids among metal atoms, electrons forming Wigner lattice, and so on) should correspond to the minimum energy configuration.

Here I would like to indicate another probable, though not yet observed, candidate for the superlattice club — namely, the isotope superlattices (ISL). The interaction between atoms of different isotopes is not exactly the same[2]. Some small (about 1 part in 1,000) differences in lattice constants of isotopically pure crystals (for example, ^{28}Si and ^{30}Si) arise because of the anharmonicity of zero-point vibrations. In an isotopically mixed crystal this could lead to a 'net' isotope interaction between atoms of the same isotope that is roughly the difference between the interaction of different isotope pairs. This will not always lead to the ordering tendency — it will depend on the particular isotope ratio. But in many cases the ordered arrangement of the minority isotope will be energetically preferable.

It is, of course, highly improbable that any natural isotope ratios will be such that the formation of ISL will occur without stoichiometric mismatch. Also, the required kinetics of crystallization should fall into quite specific margins. This probably explains why ISL have not yet been detected in natural samples. However the required isotope ratios can easily be obtained experimentally. Various solidifying liquids, such as mixtures of light and heavy water, combined with various external conditions, such as irradiation or external fields, provide an enormous variety of experimental opportunities. Although interesting in its own right, the search for isotope correlations and ISL (both natural and artificial) could also have many applications. I have recently discussed[3] the possibility of information storage based on isotope combinations.

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Amorphous definitions and glasses

SIR—In their recent paper Phillips *et al.*[1] present evidence for the existence of supernanometric microcystallites in 'amorphous' silicon. We should admit, however, that there are many ways in which a solid does not have to be a crystal: the random network model of the structure of glasses does not always give way to clusters of microcrystallites.

There is incontrovertible evidence that quartz glass has a coherence length of about 1 nm, as expected for a random network: the thermal phonon[2] mean free path at 80 K is only 1.2 nm, as compared with 70 nm in quartz crystal (parallel to the *c* axis). There is no elastic scattering process known to me that can reduce a phonon to such a short path except phase cancellation between atomic motions on adjacent arms of a random network, by which is meant that a phonon moving from atom A to atom E proceeds in part by a long path ABDE and in part by a short path ACE. Between room temperature and 100 °C the mean free path in the glass is about 0.8 nm, consistent with structural coherence of the same dimension. A structure of 3-nm microcrystallites would give proportionately longer mean free paths near room temperature, and the increase at low temperatures as we enter the Rayleigh region would be deferred to still lower temperatures.

The enormous photon extinction length of transmission-quality optical fibres is consistent with a random network model of the glasses used in the fibres. Rayleigh scattering[3] in a wide frequency region is the dominant intrinsic mechanism that limits the extinction, and here we are talking about propagation over 100 km. Rayleigh scattering is proportional to the coherence volume: a change from (0.8 nm)^3 to (3 nm)^3 would have a measurable deleterious effect.

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Lung surfactant anchor

SIR—Patthy's[1] recent correspondence regarding the nature of the protein component of pulmonary surfactant is intriguing. As he points out, the major protein in both human and canine lung surfactant seems to have a collagen-like region within its amino-terminal half and a non-collagenous globular domain within its carboxy-terminal moiety. Of particular interest is the fact that the non-collagenous domain strongly resembles a module that is present in the proteoglycan of rat chondrosarcoma, the humoral lectin of the insect, *Sarcophaga peregrina,* and the membrane-associated lectins of human, rat and chicken hepatocytes.

In a recent cytochemical study of human neonatal lungs[2], I found that the filamentous surface coating of the epithelial cells ('pneumonocytes') in the distal air spaces had binding sites for several lectins suggesting that the following carbohydrate groups are present in the filamentous material: *N*-acetylgalactosamine, *N*-acetylglucosamine, α-D-mannose, β-D-galactose and sialic acid. Another study[3] indicates that the surface coating of hamster pneumonocytes and alveolar macrophages also contains a similar range of terminal carbohydrate groups.

It is generally assumed that pulmonary surfactant is closely associated with the surface of the respiratory epithelium. The popular view, originally advanced by Pattle[4], is that surfactant molecules form a monolayer at the interface between the alveolar air and the thin layer of fluid that moistens the epithelial cells. As the lectin component of lung surfactant is similar to that derived from hepatocyte membranes and cartilage proteoglycans, it can be expected to be capable of binding to the terminal *N*-acetylglucosamine groups in the surface coating of the alveolar pneumonocytes. The extension of the filaments into the fluid subphase of the surfactant monolayer would facilitate the binding process.

Patthy[1] has raised the possibility that the lectin-like component of surfactant protein interacts with the carbohydrate constituents of the lung surfactant complex. I share this opinion and, further, suggest that carbohydrate–lectin binding may help anchor the delicate surfactant film to the surface of the respiratory epithelium.

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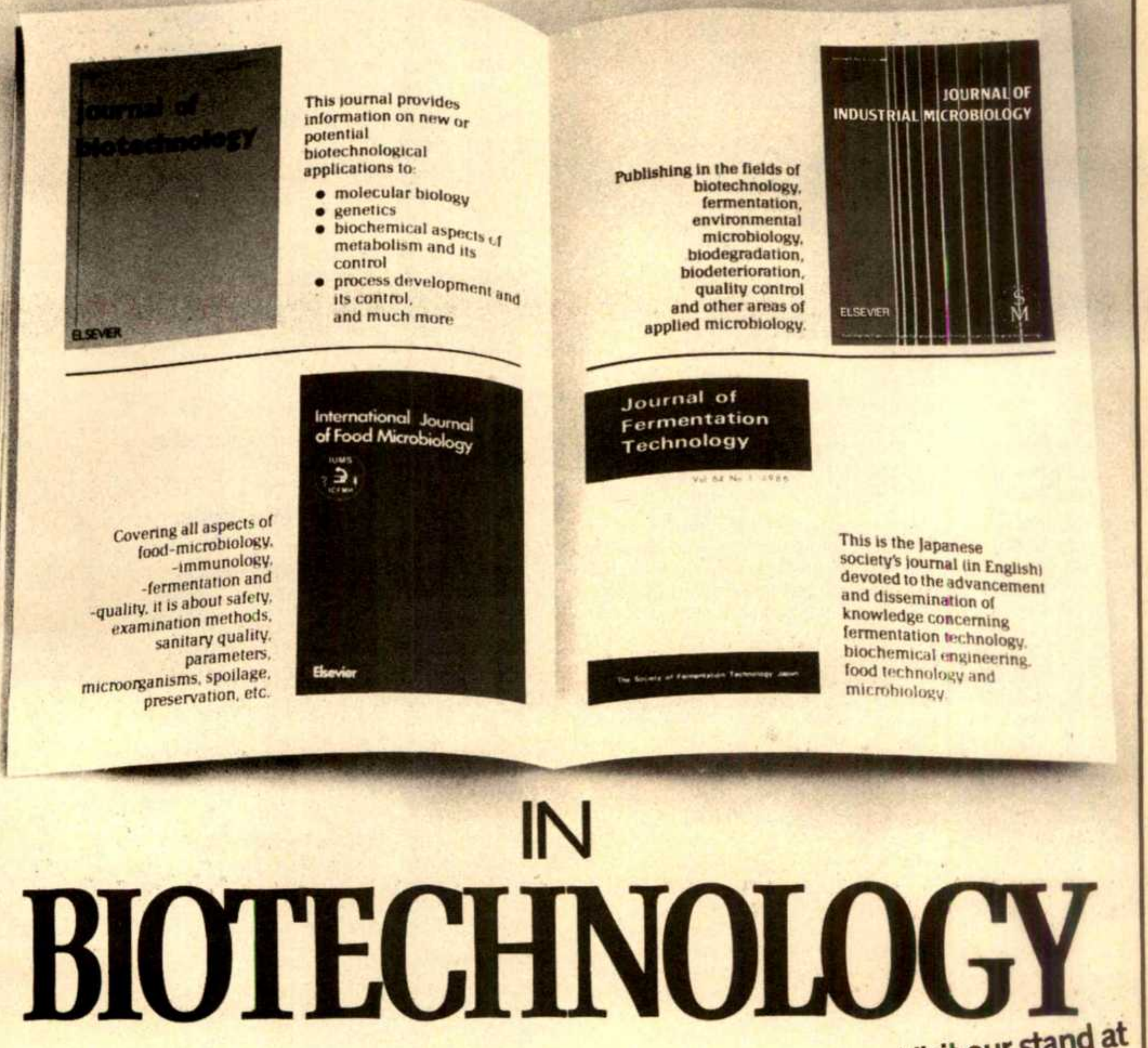
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From reviews

General

Biotechnology enables one to learn basic microbiology, biochemistry, and engineering. It also emphasizes the discipline's commercial potential by including separate chapters on patents and sources of microorganisms (very important information to those not in the field).

The editors, both of impressive scientific stature, bring strong industrial and academic backgrounds to the work. Reed, the industrialist, has had a most impressive career specializing in yeasts. Rehm, on the other hand, has spent much of his career in an academic setting and is an authority on hydrocarbon assimilation. *Bio/Technology*

Volume 1

As one might expect from such a distinguished authorship, the content and presentation of the material is of the highest calibre and draws, in the main, from recently published research material.

The present volume is admirably structured, with a good index and with very adequate references at the end of each section... *Biotech*

Volume 5

This is the most comprehensive, balanced treatment of fermented foods and beverages currently available. It is an excellent production that is both informative and stimulating to read and undoubtedly will inspire a greater interest in this challenging area of food microbiology. It will appeal to many readers and will serve as a major reference source for students, teachers and researchers in both academic institutions and industry. A contributing factor has been the choice of authors many of whom come from countries where the product of concern is of major importance.
Food Technology in Australia

Bibliographic Data

Volume 1: Microbial Fundamentals
1981. XII, 520 pages, 240 figures, 106 tables, 7 plates. Hardcover. ISBN 3-527-25763-2.

Volume 2: Fundamentals of Biochemical Engineering
1985. XVI, 819 pages, 551 figures, 132 tables. Hardcover. ISBN 3-257-25764-0.

Volume 3: Biomass, Microorganisms for Special Applications, Microbial Products I, Energy from Renewable Resources
1983. XIX, 642 pages, 190 figures, 205 tables. Hardcover. ISBN 3-527-25765-9.

Volume 4: Microbial Products II
1986. XIV, 673 pages, 244 figures, 109 tables. Hardcover. ISBN 3-527-25766-7

Volume 5: Food and Feed Production with Microorganisms
1984. XII, 631 pages, 135 figures, 201 tables. Hardcover. ISBN 3-527-25767-5.

Volume 6a: Biotransformations
1984. XII. 473 pages, 232 figures, 100 tables. Hardcover. ISBN 3-527-26768-3.

Volume 6b: Special Microbial Processes
In preparation.

Volume 7a: Enzyme Technology
In preparation.

Volume 7b: Gene Technology
In preparation.

Volume 8: Microbial Degradations
1986. XV, 744 pages, 242 figures, 121 tables. Hardcover. ISBN 3-527-25770-5.

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The stamp of greatness

W.F. Bynum

The Correspondence of Charles Darwin, Vol. 2 1837–1843. Edited by Frederick Burkhardt and Sydney Smith. *Cambridge University Press:1987. Pp.603. £30, $37.50.*
The Darwinian Heritage. Edited by David Kohn. *Princeton University Press:1986. Pp.1,138. $95, £63.40.*

THE second volume of the Darwin correspondence has now arrived, some months after the advertisements promised, but produced with the same devoted care as the first. As before, the notes helpfully explicate the letters, an appendix with manuscript variations and alterations should satisfy the most ardent Darwin pedant, and the biographical register and bibliography of books and articles mentioned make this a veritable reference guide to early Victorian natural science. The editing is by teamwork, and the product does much to restore one's faith in the committee as a means of producing works of scholarship.

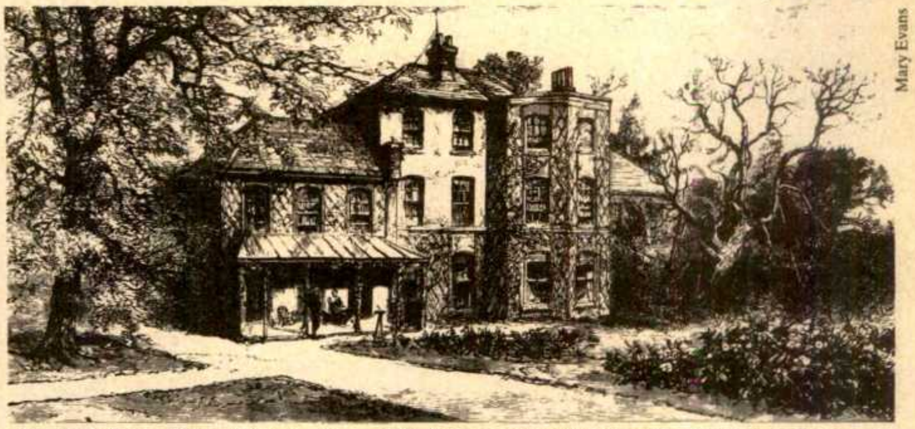
The first volume saw Darwin safely through the *Beagle* voyage. This instalment finds him establishing himself in London, before marriage and the lure of the countryside's peace and quiet prompted him and Emma to settle in what was henceforth to be their headquarters, Down House. ("House ugly, looks neither old nor new", was Darwin's initial reaction.) By the end of 1843, Darwin was an established naturalist, one of England's scientific élite, a father and, despite his 34 years, something of an old man.

It is one of the bonuses of these early volumes of the correspondence that they cover sufficiently large blocks of time to enable us to witness developments in Darwin's preoccupations, style and psychology. The contrast between the Darwin of Cambridge and the *Beagle*, and the Darwin of London and Down House, could hardly be more striking. In the present volume, Darwin ages almost before our eyes. A series of short letters to Charles Babbage are entirely given over to accepting or declining invitations to his famous parties. Darwin manages to get up the energy to go to some of these soirées, usually with a relative or house guest in tow. As often as not, however, he declines, including one splendidly frank occasion in 1838 when he writes,

> I am very much obliged to you for sending me cards for your parties, but I am afraid of accepting them, for I should meet some people there, to whom I have sworn by all the saints in Heaven, I never go out, & should, therefore, be ashamed to meet them.

Darwin's health will continue to fascinate historians, but although the complete correspondence is going to yield no definitive diagnosis, it already shows his health's relatively rapid deterioration in the late 1830s. We know from previously published correspondence that it will be a recurrent theme of the remaining volumes.

This, however, is a trivial topic compared with the substance of Darwin's scientific achievement. Appropriately, the analytical index gives one column to his health, and more than three to his 'information networks'. Ill health was simply a nuisance, for the real business of life was discovery, and these letters reveal how single-minded and ambitious the young–old Darwin was. He took infinite pains to make certain that the thousands of specimens of plants, animals, rocks and fossils that he had collected were being properly described by the best people. He slaved over his *Journal of Researches*, fretted over his theory of coral reefs, agonized over his paper on the 'parallel roads' of Glen Roy, and engaged in lengthy exchanges with Lyell, Henslow, Waterhouse, Hooker, Owen, Gould, Herbert and many others on a vast array of topics. His uncle Joshua Wedgwood was right: Darwin was supremely "a man of enlarged curiosity".


Mary Evans

Darwin's home, Down House — " . . . ugly, looks neither old nor new", was his initial reaction.

Given Darwin's concern for his place within the community of scientists, the epistolary climax for him during these years (apart, he undoubtedly would have insisted, from letters from Emma) must surely have been what for us is also the most remarkable item of the collection: the long letter of 18 September 1839 from Alexander von Humboldt evaluating Darwin's *Journal of Researches*. Writing in French about a book in English, this great German naturalist and explorer recognized in Darwin a kindred spirit. He had the advantage, of course, of having seen much of the South America that Darwin describes; nevertheless, his evaluation of Darwin's early achievement leaves the impression that Humboldt could see more clearly than any of Darwin's English contemporaries that this young naturalist was stamped not simply for distinction, but for greatness.

Humboldt died, aged 90, in that *annus mirabilis*, 1859. We now suspect that in January 1837 Darwin did not consciously believe in biological transmutation, whereas by December 1843 the basic structure that was to become the *Origin of Species* had been clearly worked out. This volume thus covers the crucial period of his scientific development. The notes to the letters occasionally point out themes and snippets of information which found their way into Darwin's notebooks, and the letters as a whole certainly make clear Darwin's intense concern, for reasons we now can understand, with animal and plant breeding. On balance, however, the correspondence reveals remarkably little of that private dialogue between himself and his notebooks. Darwin kept his own counsel for a very long time.

The various transmutation notebooks for this period have been published in several places over the past quarter of a century, but are shortly to be reissued in a single volume. This will be a great boon to Darwin scholarship, as is clear from *The Darwinian Heritage* in which the notebooks loom large. Few scientists besides Darwin would have secured such a large international group of scholars willing to contribute; even fewer might have found a publisher willing to produce a collection of articles which runs to over 1,100 pages. The 31 essays and commentaries cover most periods and themes relating to Darwin and darwinism; they can be taken as a fair reflection of the current state of Darwin scholarship, further examples of which may be found in the 70-page bibliography.

So much has been published on Darwin of late that new ideas can become scarce and 'scholarship' can be mistaken for the academic equivalent of ghetto economics (everyone earning a living by taking in each other's dirty laundry, or commenting on each other's work, as the case may be). Only Darwin enthusiasts or compulsive reviewers are likely to read all of the essays in *The Darwinian Heritage*, but those who neglect the volume entirely will miss some sound research and fine writing. My own collection would be half the size, but it would certainly include Sloan on Darwin's invertebrate research, Browne on blushing, Kottler on natural selection, Rachootin on bones, Secord on breeding and Mayr on Darwin's 'five theories'.

In the end, however, the essays are secondary literature, the correspondence is primary. Reading Darwin's correspondence may be more illuminating than reading about him. And on that scale, Darwin's scientific works stand even higher. Like *Middlemarch*, *The Origin of Species* will repay reading each year, from beginning to end. □

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Symmetrical work

Paul K. Townsend

Supersymmetry: A Decade of Development. Edited by P.C. West. *Adam Hilger: 1986. Pp.483. £25, $55.*

Supersymmetry, Superfields and Supergravity: An Introduction. By Prem P. Srivastava. *Adam Hilger: 1986. Pp.162. Hbk £25, $55; pbk £12.50, $28.*

Introduction to Supersymmetry and Supergravity. By P. West. *World Scientific: 1986. Pp.289. Hbk £31.80, $36.80; pbk £18.90, $21.85.*

Introduction to Supersymmetry. By Peter G.O. Freund. *Cambridge University Press: 1986. Pp.152. £20, $34.50.*

Unification and Supersymmetry: The Frontiers of Quark–Lepton Physics. By Rabindra N. Mohapatra. *Springer-Verlag: 1986. Pp.309. DM84, $34.*

Considerations of symmetry are central to much of modern theoretical physics; certainly, symmetry was the guiding principle behind the achievements of elementary particle physics in the 1960s. In the early 1970s it was realized that quantum field theories may possess a new and radically different kind of symmetry — 'supersymmetry'. After an initial phase in which it was seen as something of a mathematical curiosity, supersymmetry caught the imagination of increasing numbers of theoretical physicists and underwent rapid development. In 1976 a supersymmetric generalization of general relativity, 'supergravity', was found. This gave rise to new hopes of a unification of gravitation and elementary particle physics.

Ten years and many man-hours later these hopes are still alive but in the form of 'superstring' theory. The spotlight on supergravity has passed on and it is now time to write a book about it. This is the premise of at least one of these five books on the subject. *Supersymmetry: A Decade of Development* is a collection of invited essays by means of which the editor has tried to achieve a "balanced view" of the subject. The first contribution, by Golfand and Likhtman, can be recommended to those interested in the history of supersymmetry. These authors were early pioneers but their original paper (published in 1971) is hard to read, their methods being very different from those that followed, so the inclusion of this explanatory essay is all the more welcome.

The developments of the following "decade" (really 15 years) were prompted not by experimental support for supersymmetry but by the combination of its aesthetic appeal and the sheer difficulty of solving certain theoretical problems without invoking supersymmetric principles. In fact, so severe are the constraints imposed by supersymmetry that one of the considerable achievements of the period was the construction of supersymmetric theories that are not actually in conflict with experiment. These realistic models, as reviewed in the essay by G. Ross, do yield interesting new predictions but we shall have to wait for the next generation of particle accelerators to test them. Meanwhile, supersymmetry continues to find applications in other fields, such as cosmology and mathematics, the subjects of the two concluding essays. The other contributions are mostly expositions of the technical tools of the trade, many of them well-written. This is an excellent book that physics libraries would do well to acquire but which, by its nature, is not so suitable as a text for the individual student.

Because of its length, organization and choice of topics, P.P. Srivastava's *Supersymmetry, Superfields and Supergravity: An Introduction* invites comparison with *Supersymmetry and Supergravity* by Julius Wess and Jonathan Bagger (Princeton University Press, 1983). Both cover similar material on supersymmetry, but differ in their approaches to supergravity. Srivastava opts for the 'component' approach which is less geometrical than the superspace approach of Wess and Bagger but is more easily grasped on a first encounter and more easily explained. Even so, the 18 pages allotted are insufficient to do justice to the subject and this book really has to be considered as an introduction to supersymmetry alone. As such it does a reasonable job, with plenty of problems for the student at the end of each chapter.

A more detailed and comprehensive account of supergravity is to be found in *Introduction to Supersymmetry and Supergravity* by P. West, which is about a hundred pages longer. The organization is also different in that both supersymmetry and supergravity are first discussed to some depth in the 'component' approach: only then is superspace introduced and the superfield formulation presented. For supergravity, at least, this is the historical route. Whether or not it is the best one, the book is the only one currently available that deals with both formulations adequately. Some material of more recent interest, on two-dimensional models and string theory, is included in the final chapters, but these are subjects that are rapidly evolving and have already moved on to the point that the chapter on strings, in particular, seems premature.

P.G.O. Freund's *Introduction to Supersymmetry* is refreshingly unconventional. Many traditional topics are left out (super-Feynman rules for example) and the treatment of supergravity is skimpy, but to compensate there is discussion of many other things such as Lie superalgebras and eleven-dimensional supergravity. This is also the only one of the volumes under review to present some of the mathematics needed for a rigorous definition of superspace. There is an entire book on this subject (Bryce DeWitt's *Supermanifolds*, published by Cambridge University Press in 1984), but Freund's brief discussion is likely to satisfy the needs of most physicists. His book can be recommended to those looking for a brief and not overly technical exposition of supersymmetry.

Unlike the other four books, *Unification and Supersymmetry* by R.N. Mohapatra is not a text on supersymmetry *per se*. It is rather an attempt to chart the course of theoretical elementary particle physics since the electroweak unification of the late 1960s. The book starts with an exposition of the basic ideas behind the electroweak theory and proceeds, for about two-thirds of its length, through topics such as CP-violation, 'grand-unification' and 'technicolour'. There follows an account of the applications of supersymmetry and supergravity to particle physics models. Despite its brevity, the choice of material here is sufficiently different to make this a useful book on supersymmetry. It is only a pity that the author does not discuss the more recent supergravity models that are currently favoured as 'low-energy' manifestations of superstring theories. □

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Black holes made easy

William H. Press

Black Holes: The Membrane Paradigm. Edited by Kip S. Thorne, Richard H. Price and Douglas A. Macdonald. *Yale University Press: 1986. Pp.367. Hbk $37.50, £37.50; pbk $12.95, £9.95.*

WHERE are the black holes? Have they gone the way of steady-state cosmology and phlogiston, or are they still an essential element of contemporary astrophysical understanding?

The answer is that black holes, of about 10^8 solar masses, are alive and well and living in quasars and active galactic nuclei. This blunt statement is accepted by most researchers in the field, but it has been unexpectedly difficult to prove by any single, direct observation. Rather, the past decade has seen the continuing accumulation of data and theoretical understanding consistent with the black-hole model, and not very consistent with any other model. These advances may no longer have the ability to grab popular headlines (or stampedes of PhD students), but steady progress is punctuated by elegant new insights and syntheses. One of the most fruitful of them is the 'membrane viewpoint', developed by Kip S. Thorne and his collaborators, several of whom are contributors to this book.

The idea is this: Suppose we are willing to restrict our study to the interaction of already formed black holes with the external, astrophysical universe, putting aside for another book questions of black-hole formation and interiors. Then we want to think of the black hole not as a 'spacetime', but as an 'object', a three-dimensional approximately spherical 'thing' that behaves like any other astronomical body — accretes material, interacts with external matter and electromagnetic fields, and so forth. The surface of this object can be taken to be a timelike surface, a membrane, just outside (in a sense that can be made mathematically precise) the actual event horizon of the black-hole spacetime. The game now is to 'hide' the inside of the black hole by attributing all its properties to fictional, but precisely definable, properties of the membrane.

Physically motivated thought-experiments are conducted to investigate the properties of the membrane, its electrical conductivity for example. This can be discerned in several ways, say by calculating the membrane's absorbtion of electromagnetic radiation, or its Ohmic losses in a time-varying magnetic field, or from the details of how the electric field on the membrane becomes uniform if a charge is dropped onto (into!) one point. Amazingly, all such calculations give the same answer for the conductivity. The deep reason is that the exact, fully relativistic equations for electromagnetism in a black-hole spacetime can be cast into a form *exactly* like ordinary Maxwell's equations in the presence of a conducting body.

This general idea works not only for the hole's electrical properties, but for its mechanical and thermodynamic properties as well. The membrane has a viscosity that gives rise to dissipation whenever an orbiting object raises a tidal bulge — just like the Moon's tidal dissipation in the oceans of the Earth. The membrane has an entropy and a temperature, in terms of which the black hole and its surroundings can be understood as exactly satisfying the first and second laws of thermodynamics. Once again, the analogies are not merely suggestive, the are *exact* when the equations are properly cast. The membrane viewpoint is a powerful formalism, not only for actual calculations of black-hole behaviour, but also for intuitive and qualitative understanding of a hole's behaviour in the complex astrophysical environment at the centre of a quasar or active galactic nucleus.

Although the chapters in this book were written by nine separate authors, they have been polished to speak with a single voice: it is the voice of a careful teacher who, while unafraid of mathematical precision, is not satisfied with an equation until a clear physical interpretation of it has been conveyed. By virtue of its pedagogical excellence, the book transcends its particular (membrane) viewpoint and is, quite simply, about the best single work on black holes yet written. The illustrations are numerous and clear, and the index is superb. Above all, it is a book in which physics never takes a back seat to formalism. Backed up by Chandrasekhar's uncompromisingly technical *The Mathematical Theory of Black Holes* (Oxford University Press, 1983), it represents the current and maturing state of knowledge about these exotic — but not necessarily unintuitive — astronomical bodies. □

William H. Press is Professor of Astronomy and Physics at Harvard University, Cambridge, Massachusetts 02138, USA.

Of barriers and carriers

S.R. Caplan

Principles and Models of Biological Transport. By Morton H. Friedman. *Springer-Verlag: 1986. Pp. 260. DM 165.*

BOOKS on transport, especially biological transport, are two-a-penny (figuratively speaking) and at first glance it is not easy to discern why we need yet another treatise on the topic. Can there be any reason to repeat what has only just been said with great profundity in 685 pages, but this time in less than half the space?

It seems that there may be. Brevity has its advantages, especially where wordiness is replaced by precise description in mathematical terms. Because this monograph is intended to provide an introductory course for undergraduate biomedical engineers and graduate students in biophysics, the author can afford to present most of the material as an exact science, highlighting the main concepts without becoming bogged down in a mass of experimental minutiae. In this way a remarkably wide variety of topics is covered, including thermodynamics of transport processes (both the equilibrium and nonequilibrium varieties), diffusion (free and facilitated), active transport, kinetic and other models, single-cell and epithelial phenomena, gaseous processes and much else besides. Nerve, muscle and kidney all receive due attention.

Unfortunately, several caveats must be recorded. The fact that difficulties are glossed over (and important issues ignored) is less bothersome than the author's tendency to trot out often-repeated misunderstandings. Among these is the claim that the "dephosphorylation reaction" (of ATP) "is accompanied by the release of the energy stored in the terminal phosphate bond" (p.78). This actually precedes by a few pages a prominent reference to a paper by Eisenberg and Hill, wherein the surprised reader will find this view soundly debunked. Elsewhere the condition generally known as detailed balancing is said not to hold for active transport (p.101), and the reflection coefficient is indicated to lie between zero and unity (p.119).

The sternest caveat, however, relates to the inadequate and arbitrary referencing of sources. Quoting from contemporaries without citation is a serious discourtesy, which, moreover, denies the reader access to the most relevant literature. By contrast, the gentleman cited repeatedly as "van t'Hoff" might have welcomed anonymity!

Still, this is a book that will repay study by those who keep their wits about them. □

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●In his review last week (p.218), Robert Whittle expressed the hope that Wiley would issue a "more imaginatively" priced edition of *Genetic Analysis of Animal Development*. The book will appear in paperback in May; price will be £25.40, $34.95, against £66.95, $69.95 for the hardback.

Birdlife down on the farm

John Andrews

Farming and Birds. By Raymond J. O'Connor and Michael Shrubb. *Cambridge University Press: 1986. Pp.290. £17.50, $34.50.*

FARMING is the main agent of the destruction of terrestrial wildlife habitats throughout the world. Sometimes the process is quick and obvious, such as the clearance of forest or the drainage of wetland. Often it is gradual and imperceptible, for instance where domestic livestock introduced to natural grassland graze-out the palatable vegetation. At the same time, there have always been species with adaptations suited to the new circumstances and with the powers of dispersal necessary for colonization. Some birds have altered their world ranges in response to opportunities created by agriculture, many have increased in abundance and a few become economically significant pests. Where the entire land surface is man-modified, farms may retain semi-natural areas which are little used and remain relatively species-rich, while the farming system itself may sustain most or all of the populations of some species — grey partridge, rook and whitethroat in Western Europe, for example.

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In both Europe and the United States the future of agriculture is unclear, mainly because of over-production, and conservation bodies that wish to influence that future must understand and be able to demonstrate the ecological effects of different farming practices. The publication of *Farming and Birds* is therefore most timely, particularly because it is, perhaps surprisingly, the first systematic attempt to review knowledge of the impact of British agriculture on the country's birdlife. It confirms the subjective view of many naturalists that recent changes in practice have been generally detrimental to birds and hoists a warning flag over the future of several species. Moreover, its broad findings probably have relevance in all countries with technically advanced agriculture.

The senior author, Raymond O'Connor, is director of the British Trust for Ornithology (BTO) which has built up data on bird population changes for over 25 years, mostly in the form of systematic observations made by hundreds of skilled amateurs. Michael Shrubb is one such amateur and, more importantly, a farmer with a wide understanding of agricultural systems. They have drawn on four sources of data — the BTO's Common Birds Census and Nest Record Scheme, including much previously unanalysed material; the existing literature, some of it unpublished; the Ministry of Agriculture's annual farm census returns which show changes in cropping patterns; and surveys of fertilizer usage. The volume of data available was such that a 1980 cut-off date was applied.

Farming is a dynamic activity and the authors commence by summarizing its history from the Middle Ages to the Second World War, showing how patterns of grass cover and tillage change in response to economic forces. Next they describe Britain's farmland bird community, which has always been small in terms of numbers of species. The original climax vegetation being woodland, Britain has few birds adapted to life in open fields, unlike North America for instance. At the core of the farm avifauna are woodland birds which use hedges, farm woods and farmstead gardens for nesting and for much of their foraging. Their overall distribution is determined by climate, soil type and terrain, both directly and indirectly through the influence of these factors on farming procedures. The value of farmland as nesting and foraging habitat is assessed in relation to performance in primary habitats and in different farming contexts. The authors conclude that mixed farming systems, combining tillage crops and livestock within holdings averaging about 25 hectares, are most favourable to bird diversity and abundance.

Mechanization and chemical technology increasingly permit specialization and intensification in agriculture, and the effects on birdlife are considerable. A number of them are considered, including the loss of feeding opportunities associated with the reduction in the number of mixed farms; the removal of features such as hedges and ponds; the intensification of use of semi-natural farmland (such as the conversion of old permanent pasture); changes in the timing of cropping, including the shift to autumn-sown cereals; and the use of pesticides and artificial fertilizers. Several birds, common until recently, including linnet, grey partridge and lapwing, are seen as being at risk of extinction in certain regions.

Eric Hosking

*Not sitting pretty — the lapwing (*Vanellus vanellus*), which is under threat from new farming practices in certain regions of Britain.*

The book is open to criticism on several counts. There are some dangerous simplifications, such as the statement that "wildlife can adapt to change and has done so in the past but it cannot keep pace with the speed of modern technological development". A number of conclusions are tentative, because data are incomplete, or have been inferred from the fact that changes in bird numbers or distribution coincide with change in farming practice. And some studies by other workers appear to be accepted uncritically. The fact is, as the authors themselves state that knowledge of the effects of farming operations on birds is still very limited and the ecology of farmland species is understood only poorly. Nonetheless this is a valuable exercise, readable if somewhat repetitious, with many pointers for further study in Britain and elsewhere. ☐

John Andrews is Chief Advisory Officer for The Royal Society for the Protection of Birds, The Lodge, Sandy, Bedfordshire SG19 2DL, UK.

New Releases from Springer-Verlag

Hematology

Acute Leukemias

Prognostic Factors and Treatment Strategies

Editors: T. Büchner, G. Schellong, W. Hiddemann, D. Urbanitz, J. Ritter

1987. 215 figures, 212 tables. Approx. 740 pages. (Haematology and Blood Transfusion, Volume 30). Soft cover DM 218,–. Reduced price for the subscribers of the journal "Blut" Soft cover DM 174,40. ISBN 3-540-16556-8

This book presents the results of the major multicenter trials in adult and childhood acute leukemias. Topics covered include biology, cytogenetics and morphology in acute leukemias, myeloid leukemia, lymphoblastic leukemia and bone marrow transplantation.

Immunology

Pediatric Dermatology

Advances in Diagnosis and Treatment

Editors: R. Happle, Nijmegen, **E. Großhans,** Strasbourg

1987. 103 figures, 45 tables. IX, 196 pages. Soft cover DM 86,–. ISBN 3-540-16991-1

Contents: Genodermatoses. – Immunodermatology of Childhood. – Irritant Dermatitis and Eczema. – Skin Tumors. – Hair and Nails. – Subject Index.

Systemic Lupus Erythematosus: Clinical and Experimental Aspects

Editors: J. S. Smolen, C. C. Zielinski, University of Vienna

1987. 58 figures, 44 tables. Approx. 370 pages. Hard cover DM 198,–. ISBN 3-540-17154-1

This volume covers questions related to the history, etiology, pathogenesis, clinical aspects and therapy of systemic lupus erythematosus (SLE). Both animal models and human SLE are considered. Concise information on cellular immunology, autoantibodies, viral aspects and molecular biology in SLE is provided. Clinical topics then deal with medical, dermatologic, neurologic, radiologic, pathologic, and therapeutic aspects.

Oncology/Pathology

Renal and Adrenal Tumors

Pathology, Radiology, Ultrasonography, Therapy, Immunology

Editors: E. Löhr, L.-D. Leder, University of Essen

2nd, completely revised edition. 1987. Approx. 229 figures (7 in color) in 376 separate illustrations. Approx. 340 pages. Hard cover DM 298,–. ISBN 3-540-16554-1

In this new, second edition on diseases of the kidney and adrenal glands, research on pathological anatomy forms a solid platform from which the multiplicity of renal and adrenal tumors are discussed. Aspects as diverse as radiology, pathology, urology, pediatrics and immunology have been brought together in one book. The most up- to-date methods of tumor diagnosis by CT, NMR, and ultrasound are covered, as are methods of catheter embolization and radiation techniques in case of primarily inoperable tumors.

T. Nakashima, M. Kojiro, Kurume University

Hepatocellular Carcinoma

An Atlas of Its Pathology

Foreword by H. Popper

1986. 368 figures, mostly in color. XII, 267 pages. Hard cover DM 390,–. ISBN 3-540-70018-8

This instructive atlas presents the authors' accumulated pathomorphological knowledge of hepatocellular carcinoma, based on the examination findings of 439 cases autopsied at their department in the last twenty years.

Hyperthermia and the Therapy of Malignant Tumors

Editor: C. Streffer, University of Essen

1987. 52 figures, 62 tables. approx. 210 pages. (Recent Results in Cancer Research, Volume 104). Hard cover DM 148,–. ISBN 3-540-17250-5

Contents: Heat Delivery and Thermometry in Clinical Hyperthermia. – The Biological Basis for Tumor Therapy by Hyperthermia and Radiation. – Physiological Effects of Hyperthermia. – The Combination of Hyperthermia and Radiation. – Clinical Investigations. – Hyperthermia and Drugs. – Subject Index.

Mouse Liver Tumors

Relevance to Human Cancer Risk

Symposium of the European Society of Toxicology held in Rome, February 2-5, 1986

Editors: P. L. Chambers, University of Dublin; **D. Henschler,** University of Würzburg; **F. Oesch,** University of Mainz

1987. 64 figures. VI, 295 pages. (Archives of Toxicology, Supplement 10). Soft cover DM 142,–. ISBN 3-540-17124-X

This conference of the European Society of Toxicology addressed the puzzling question of why the mouse liver is more prone to "spontaneous" tumors than many other organs or the liver of other species. The problems are dealt with from the biological and pathological manifestations to the molecular basis, with respect to carcinogen metabolizing enzymes, DNA damage and its repair, as well as to the role of oncogenes in spontaneous liver tumorigenesis in mice. Modifying factors such as diet, ageing, and hormones are considered.

Progress in Surgical Pathology

Volume VI

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Seismological measurement of stellar ages

Douglas Gough

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Accurate measurements of stellar acoustic oscillation frequencies, which are likely to be obtained in the coming years, will provide important new data enabling us to diagnose the internal structure of stars. The ages of main-sequence stars are likely to be determined to a higher precision than has been possible in the past and much more accurate estimates of the total heavy-element abundances should be possible.

WE shall never have from the stars a wealth of seismic data such as has been gathered from the Sun[1]; variations across the surface of a distant star cannot be well resolved, and therefore only those modes of oscillation with low degree l can be detected. (The spatial variation of the vertical velocity and optical intensity fluctuations associated with a mode of oscillation is proportional to a spherical harmonic of degree l.) An impression of what might be obtained is illustrated in Fig. 1, which is a temporal power spectrum of the line-of-sight (Doppler) frequency shift of the 769.9 nm K line in light that has been spatially integrated over the entire disk of the Sun, as would be light from the stars. Only modes with $l \leq 3$ are evident[2]. Their discrete cyclic frequencies $\nu_{n,l}$ are approximated by the asymptotic formula[3], valid when $n \gg l$:

$$\nu_{n,l} \sim (n+\tfrac{1}{2}l+\tilde{\alpha})\nu_0+\varepsilon_{n,l} \qquad (1)$$

where n is the order of the mode, $\tilde{\alpha}$ is a constant of order unity, and $\varepsilon_{n,l}=O(\nu_0 n^{-1})$ is small compared with $\nu_{n,l}$; in the figure $n' \equiv n+\frac{1}{2}l$ lies between 13.5 and 28. The constant ν_0 is a characteristic frequency of the star, defined as the harmonic mean with respect to radius r of the speed of sound $c(r)$ divided by the diameter of the star.

As indicated by equation (1), the peaks in the power spectrum are grouped in pairs, the groups being separated by a frequency interval $\Delta_{n'} \simeq \frac{1}{2}\nu_0$. Furthermore, modes of a group with like n' are separated in frequency by $d_{n,l} \equiv \varepsilon_{n,l}-\varepsilon_{n-1,l+2}$. With the theoretical knowledge that $d_{n,l} \propto 2l+3$ and that $d_{n,l}>0$, it is possible to ascertain whether modes in a group have odd or even l, and then to identify the value of l pertaining to each of the peaks in the power spectrum.

The modes under discussion have substantial amplitudes throughout most of the interior of the star; they avoid merely a central sphere, which for solar-like stars has radius $r_t \simeq 0.8\,(l+\frac{1}{2})R/(n'+\tilde{\alpha})$, where R is the radius of the star. Thus some of them penetrate into the central core where nuclear reactions are taking place.

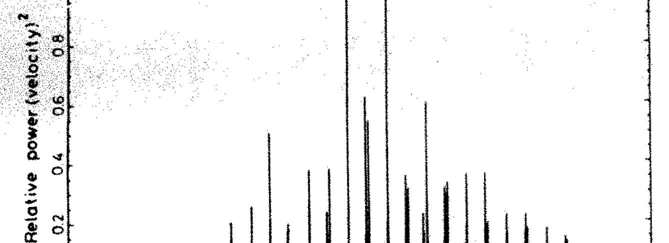

Fig. 1 Power spectrum of 88 days of nearly continuous observations of whole-disk spectrum line shifts obtained from stations in Tenerife and Hawaii, which are separated by 9.7 h in longitude (after ref. 17). The maximum amplitude = 15 cm s^{-1}.

Acoustic modes can be regarded as a superposition of sound waves which propagate between the stellar surface and the radius r_t at which downward directed waves are refracted back towards the surface. When $n \gg l$ their frequencies form an approximately harmonic sequence which depends on the size of the acoustic cavity within which the waves propagate (from $r=r_t$ to $r=R$), and on the (harmonic) mean of the speed of sound c within that cavity. Thus, because $r_t \ll R$, the cavity is almost the entire star and therefore the frequency interval $\Delta_{n'}$ depends only weakly on the value of r_t, and hence only weakly on n'; it is approximately $\frac{1}{2}\nu_0$, as is evident from equation (1), which is a global average of c. The separation $d_{n,l}$ on the other hand, which measures the extent to which the overtones deviate from an harmonic sequence, is the difference between two similar averages of c and is therefore dominated by conditions between the two values of r_t associated with the modes whose frequencies are differenced. Thus $d_{n,l}$ depends most sensitively on conditions in the core.

As a star evolves on the main sequence, hydrogen is converted into helium in the core, which increases the mean 'molecular weight' of the stellar material near the centre of the star and decreases the sound speed. As a result of this variation alone, acoustic frequencies are reduced. The reduction is greater for modes with smaller l, which penetrate more deeply into the core. Therefore $d_{n,l}$ is reduced. The dominant response of the nonreacting envelope of the star is to expand. The size of the acoustic cavity is increased, and consequently Δ_{n} is reduced too.

The structure of a main-sequence star of given initial chemical composition depends only on its mass M and age t. Therefore, in principle, it should be possible to determine both these quantities from a knowledge merely of representative, though well defined values of $\Delta_{n'}$ and $d_{n,l}$. Indeed, as a first approximation, zero-age lower main-sequence stars, for example, may be considered to form an homologous sequence satisfying $M \propto R^{3/4}$. Under homologous transformation the overtone frequency interval satisfies the 'pendulum formula': $\Delta_{n'} \propto (g/R)^{1/2} = (GM/R^3)^{1/2}$, where g is the surface gravity, and is proportional to the square root of the mean density of the star. Therefore $\Delta_{n'} \propto M^{-3/2}$, a result which is confirmed by numerical computation[4,5]. As the star subsequently evolves its radius increases and the homology scaling continues to hold: $\Delta_{n'} \simeq 135(M/M_\odot)^{1/2}(R/R_\odot)^{-3/2}$ μHz. Thus $\Delta_{n'}$ depends on both mass and age. Notice, however, that surface gravity g can be estimated spectroscopically, and together with $\Delta_{n'}$ can be used to determine M and R separately. The separation $d_{n,l}$ measures the nonhomologous change in the core, and therefore determines principally what one might call the evolutionary age $\tau = t/t_{ms}$,

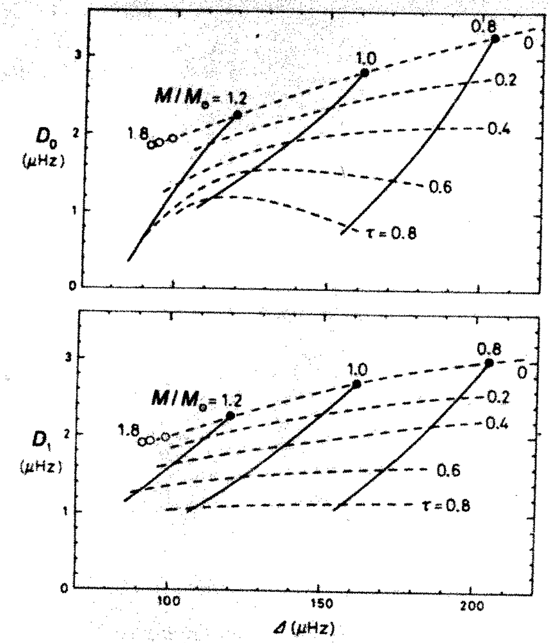

Fig. 2 Continuous lines are contours of constant M, dashed lines are contours of constant τ, estimated from theoretical data in the frequency range 1.5–3.8 mHz provided in refs 5 and 13 for main-sequence stellar models with $Y = 0.257$ and $Z = 0.017$. The dashed curves, in particular, were obtained by interpolation between points on the continuous curves, and in reality may vary more sharply than depicted in the bottom left-hand corner of the upper diagram where it is evident that the frequencies of the lowest-degree modes are influenced by the convective core that develops in stars somewhat more massive than the Sun. Physical time is $t = t_{ms}\tau$; the values of t_{ms} are 22.8, 9.1 and 4.3×10^9 yr for $M = 0.8$, 1.0 and 1.2 $M_\odot$ respectively. The circles on the zero-age contours are at intervals of 0.2 $M_\odot$ in mass; filled circles were otained from refs 5 and 13, and open circles, which are representative values for both $l = 0$ and $l = 1$ in the frequency range 2-4 mHz, from ref. 4.

where I define t_{ms} to be twice the time taken for the central hydrogen abundance to be reduced to half its initial value. Of course t_{ms} depends on M, and for solar-type stars $t_{ms} \propto M^{-4}$. Hence, even though $d_{n,l}$ might be expected to depend relatively weakly on M at fixed τ, it is necessary to know both τ and M (and thus both $d_{n,l}$ and $\Delta_{n'}$) to ascertain the absolute age t.

Seismic measurements of lower main-sequence stars have not yet attained adequate sensitivity for measuring $d_{n,l}$. Indeed, individual modes of oscillation have not even been isolated. Nevertheless, Fossat *et al.*[6,7] and Noyes *et al.*[8] have between them searched for oscillations in three late-type main-sequence stars, α Centauri A, Procyon and ε Eridani, and have found statistical evidence for modes whose frequencies satisfy equation (1). They have been able to estimate only ν_0 from their observations, and therefore the astrophysical inferences that have so far been made rely heavily on other astronomical evidence[9-11].

Isolating the modes in a power spectrum such as Fig. 1 requires long, almost continuous data sets; under ideal conditions it would be necessary to observe for about two weeks to attain a precision of 1 μHz, for example, in the measurement of $d_{n,l}$. Several groups of astronomers are planning such measurements, either by coordinated observations from two or more ground-based sites around the world, or by observing from space.

In anticipation of imminent progress, Christensen-Dalsgaard[4] has carried out the first computations to quantify the theoretical ideas I have outlined above. In particular, he has plotted for several values of M at $t = 0$ and for several values of t at $M = M_\odot$ a representative value (which he has called D_0) of $D_{n,l} = d_{n,l}/(6+4l)$, which is asymptotically independent of l, against a representative value ($\Delta\nu_0$) of $\Delta_{n'}$. Both quantities were obtained by fitting numerically computed eigenfrequencies to the asymptotic formula (1). The result confirmed that, at least for stars with masses similar to that of the sun, contours of constant M and constant t are not even nearly parallel (see for example, Fig. 2), and therefore it appears that in principle it would indeed be possible to determine both M and t from $D_{n,l}$ and $\Delta_{n'}$. More detailed results[12] were presented at the IAU Symposium 123 on Advances in Helio- and Asteroseismology held in Aarhus last July.

Results similar to those of Christensen-Dalsgaard are illustrated in Fig. 2, where contours of constant M and τ are plotted against representative values D_l and Δ of $D_{n,l}$ and $\Delta_{n'}$. They were derived mainly from recent computations by Ulrich[5,13]. It appears that in principle either one of the plots could be used to determine M and τ simultaneously from Δ and either D_0 or D_1; I present both diagrams to show how the seismic data contain even more information. In particular, the relatively high curvature of the isochrones in the bottom left-hand corner of the Δ-D_0 plane is associated with the development of convective cores in the more massive stars. It is important to realise, however, that the determination of M and τ could be accomplished by comparing observations with these diagrams only if the chemical composition, and the mixing-length parameter α used in modelling the convection zone, can also be ascertained. That requires the use of additional astronomical data.

The structure of theoretical stellar models depends on M, t, α and chemical composition, which is usually characterized by any two of the parameters X, Y and Z, the initial relative abundances by mass of respectively hydrogen, helium and all other elements ($X + Y + Z = 1$; the relative abundances of the heavy elements that influence stellar structure most strongly is usually assumed to be known[14].) For given X, Z and α, stellar evolution theory relates the luminosity L and the surface effective temperature T_e to M and t. Thus if L, T_e and say Z/X are determined astronomically, as might be expected for stars in clusters, the seismic parameters Δ and D_0, say, are then formally sufficient to determine M, t, Y and the mixing-length parameter α. If the star is one of a binary pair, so that M is also known, one would then expect it to be no longer necessary to know the chemical composition. But how accurate would the determinations be?

A step towards answering that question has been made in two recent papers by Ulrich, which address separately the seismological determination of mass[13] and age[5]. By combining the results from the two papers one can estimate the sensitivity of the determinations to errors in the data. An error matrix for a solar-type star is presented in Table 1, which lists the partial derivatives of t/t_{ms}, $\ln M$, $\ln Y$ and $\ln \alpha$ with respect to the parameters listed in the first column; the units of these parameters are stated in parentheses, and represent perhaps somewhat optimistic estimates of the precision with which the parameters can be measured. The most striking feature is the high sensitivity to the relatively poorly known heavy-element abundance Z, which, even ignoring any doubts one might have about assuming the value of Z in the stellar interior to be the same

Table 1 Formal errors in inferred age t, mass M, initial helium abundance Y and a mixing-length parameter α

	$\delta t/t_{ms}$	$\delta M/M$	$\delta Y/Y$	$\delta\alpha/\alpha$
T_e (2%)	−0.06	0.04	−0.09	0.16
L (10%)	0.03	0.05	−0.02	−0.11
Z/X (50%)	1.1	−1.9	5.6	−2.6
Δ (1 μHz)	0.01	0.01	−0.02	−0.003
d_0 (1 μHz)	−0.13	−0.02	0.10	−0.03

Formal errors for a solar-type star resulting separately from errors in the measurements of T_e, L, Z/X, Δ and d_0 (of the magnitudes stated in parentheses) computed from the data in refs 5 and 13. The parameter d_l is $(6+4l)D_l$, and is thus a representative value of $d_{n,l}$. The effect of replacing d_0 by d_1 is to multiply each element of the last row by approximately 3/5; the remaining elements are hardly changed.

Table 2 Transformation of the error matrix of Table 1, with g replacing Z/X

	$\delta t/t_{ms}$	$\delta Z/Z$	$\delta Y/Y$	$\delta\alpha/\alpha$
T_e (2%)	0.04	0.07	0.12	−0.005
L (10%)	−0.03	−0.02	−0.24	−0.008
g (20%)	−0.17	−0.07	−0.57	0.28
Δ (1 μHz)	0.01	0.01	−0.002	−0.013
d_0 (1 μHz)	−0.14	−0.04	0.05	−0.003

as that in the atmosphere, renders it impossible to determine either M or t. This conclusion is at variance with the view advanced by Ulrich.

Use of additional, more subtle features of the frequency spectrum or independent measurements of other stellar parameters could permit the determination of t. In particular, the situation is eased substantially by using a separate estimate of M, either indirectly from the surface gravity g, or directly if the star is a member of a binary system. In this case, T_e and L determine R, so M/R^3 can be estimated directly and the role of Δ, like d_0, is now solely to provide information about deviations from homologous variation. The outcome is illustrated in Table 2, which is the error matrix for the case when g is measured. In practice, spectroscopic measurements actually estimate combinations of quantities such as T_e, L, g, Z/X and Y, and it is a straightforward matter to transform the error matrix for any given self-consistent complete set of such combinations.

It is evident from Table 2 that one can now hope to know the age of a solar-type star to within perhaps 20% of its main-sequence lifetime t_{ms}. Moreover, one can also measure Z, which determines the opacity in the interior of the star. It would therefore be particularly interesting to make seismological observations of a metal-deficient late-type star; B. E. J. Pagel (personal communication) has suggested ν Indi, which is a fifth magnitude G star in the Southern Hemisphere. Unfortunately, according to Table 2, without additional data one cannot determine Y to useful accuracy.

It should be appreciated that the measurement of t and Z is based on a calibration of theoretical models, and therefore depends on the accuracy of those models. Therefore one must heed the fact that it has not yet been possible to reproduce accurately the seismic data from the Sun by adjusting the parameters of the theory; until we truly understand why that is so we must treat any calibration of stellar models with due caution. In the solar case, the slight, possibly insignificant discrepancy[15,16] between the observed and calculated values of D_0, for example, might imply that the Sun is somewhat older than the 4.7×10^9 yr that is generally presumed; alternatively, there may be some important physical process that has not been adequately described by the standard theory. Nevertheless, it is evident that any seismic data we do obtain from stars is likely to improve our knowledge of those stars considerably.

I thank M. G. Edmunds, J. W. Harvey, B. E. J. Pagel and M. J. Thompson for useful conversations.

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ARTICLES

A genetic pathway for the specification of the vulval cell lineages of *Caenorhabditis elegans*

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Twenty-three genes have been assigned to particular steps in a genetic pathway for the specification of the vulval cell lineages of the nematode Caenorhabditis elegans. *Mutations in most of these genes cause homoeotic transformations in the fates of individual cells, suggesting that these lineages may be specified by a series of decisions that distinguish between alternative cell fates. Fifteen of the genes function in a system involved in the intracellular response to the extracellular signal that induces vulval formation.*

To understand how patterns of cell divisions and cell fates are specified during development, the complete cell lineage of the nematode *Caenorhabditis elegans* has been determined and extensively analysed[1-8]. We have focused our attention on the cell lineages involved in vulval development for three main reasons. First, the vulval cell lineages comprise only three rounds of cell division that occur over a period of only five hours[1]. Second, the specification of the vulval cell lineages appears to involve both cell-autonomous and non-cell-autonomous decisions[5-8]. Third, certain mutants abnormal in the vulval cell lineages can be recognized easily with a dissecting microscope and are viable as homozygotes[9-12].

So far, we have identified more than 100 mutants that have one of two phenotypic abnormalities in vulval morphology. Multivulva (Muv) mutants have multiple vulva-like protrusions along their ventral sides; vulvaless (Vul) mutants lack a vulva. We believe that we have identified most genes for which lack of gene function results in a Muv or Vul phenotype (see ref. 12 for discussion). Most of the genes defined by Muv or Vul mutations have been named *lin*, for lineage abnormal.

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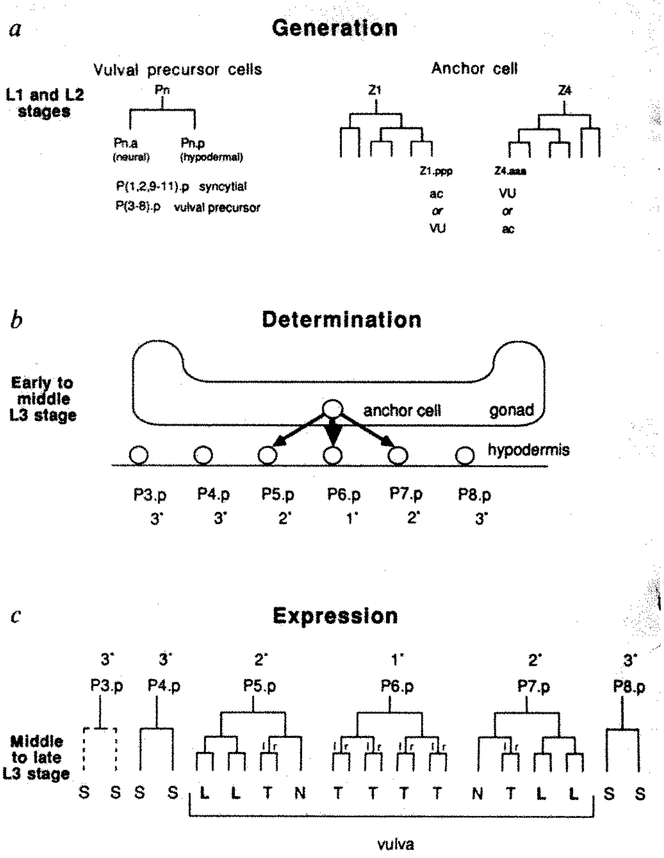

Fig. 1 Developmental pathway for the vulval cell lineages (based on refs 1, 5, 7 and 8). *a*, Generation of the seven cells involved in vulval development, P(3–8).p and the anchor cell (ac) (see text). In these diagrams, each vertical line represents a cell and each branch represents a cell division. Divisions are anterior–posterior, unless otherwise indicated; the anterior daughter is indicated by the left branch. The cells involved in vulval development are named according to their ancestries; for example, P6.p is the posterior daughter of P6, and Z4.aaa is the anterior daughter of Z4.aa, which is the anterior daughter of the anterior daughter of Z4. (See ref. 1 for a description of the conventions used in drawing cell lineages and in naming cells.) Pn, any of the 12 ectodermal progenitor cells P(1–12). Z1 and Z4, the two somatic gonadal progenitor cells. ac, the anchor cell. VU, a ventral uterine progenitor cell. *b*, Determination of the fates of the vulval precursor cells. During the early to middle L3 stage, a graded inductive signal (indicated by the arrows) from the gonadal anchor cell specifies which of three lineages (1°, 2°, or 3°) each of the six tripotent precursor cells P(3–8).p will express (see text). *c*, Expression of the vulval lineages. During the middle to late L3 stage, P(3–8).p express their respective lineages. P(3,4,8).p express a 3° lineage; these cells divide once and join the hypodermal syncytium. Each cell that joins the hypodermal syncytium is represented by [S], so the 3° lineage is represented by [S S]. (In ~50% of wild-type hermaphrodites, P3.p joins the hypodermal syncytium without dividing; this alternate fate is represented by [S].) P(5–7).p divide to generate vulval cells. The lineage of each of these cells is represented by a set of four letters. Each letter indicates the fate of one of the granddaughters of P(5–7).p, referred to as a P(5–7).pxx cell. (An 'x' after a cell name refers to both daughters of that cell, for example the P5.px cells are P5.pa and P5.pp, the anterior daughter and the posterior daughter, respectively, of P5.p.) P6.p expresses a 1° lineage, which is represented by [TTTT] because the four P6.pxx cells (formed by two rounds of anterior–posterior divisions) all divide transversely (left, l and right, r). P5.p and P7.p, which express 2° lineages, undergo polar lineages, represented by [**LL**TN] and [NT**LL**], respectively; the lineage of P5.p is mirror-symmetric to that of P7.p. After P5.p undergoes two rounds of anterior–posterior divisions, each P5.pax cell divides longitudinally, [**L**]; P5.ppa divides transversely, [T]; and P5.ppp fails to divide, [N]. The lineage of P7.p is analogous. During the late L3 and early L4 stages, the anchor-cell-proximal cells, the descendants of P5.pp, P6.p and P7.pa (all cells formed by [T] or [N] lineages) detach from the ventral cuticle and move dorsally forming a vulval cavity (see ref. 8). The anchor-cell-distal cells, the descendants of P5.pa and P7.pp (all cells formed by [**L**] lineages) adhere to the ventral cuticle. (The character of adherence is indicated by the bold-face type of the '**L**'). The anchor cell nucleus subsequently disappears and, at the L4 molt, certain of the invaginated cells (all daughters of P5.pp and P7.pa) move first toward the centre of the vulval cavity and then ventrally.

Here we report the vulval cell lineage defects resulting from representative alleles of genes defined by Muv and Vul mutants and assign 23 of these genes to specific steps in our recently-proposed pathway[8] of wild-type vulval development.

Wild-type vulval development

A model for the pathway of development of the vulva of the wild-type *C. elegans* hermaphrodite is shown in Fig. 1[1,5,7,8]. The vulva is formed by the descendants of three of six equipotent cells of the ventral hypodermis, in response to an inductive signal from the anchor cell of the somatic gonad. Vulval development consists of three temporally distinct steps (described in more detail below): the generation of the seven cells involved in vulval development, the determination of the fates of the equipotent vulval precursor cells in response to the anchor cell signal, and lastly the expression of each of these fates.

(1) Generation. The six cells with the potential to express vulval cell lineages, called P(3–8).p, are the posterior daughters of six of the twelve P cells. The nuclei of the twelve P cells migrate from a ventro-lateral position into the ventral cord during the middle of the first larval (L1) stage. Subsequently, each P cell divides to produce an anterior daughter (a Pn.a neuroblast) and a posterior daughter (a Pn.p hypodermal cell) (see Fig. 1*a*, which also explains the notation). Five of the Pn.p cells, P(1, 2, 9–11).p, fuse with the hypodermal syncytium during the L1 stage, but P(3–8).p, the 'vulval precursor cells', do not fuse. The gonadal anchor cell is formed during the second larval (L2) stage by either of two bipotent cells, Z1.ppp or Z4.aaa (Fig. 1*a*); interactions between these two cells cause one to become the anchor cell (ac) and the other to become a ventral uterine progenitor (VU) cell.

(2) Determination. Each of the six vulval precursor cells P(3–8).p is able to express any of three lineages (called 1°, 2° or 3°), each of which is defined by a distinct pattern of cell divisions (see below). During the early to middle third larval (L3) stage a graded signal from the gonadal anchor cell induces vulva formation and specifies which of the three lineages each of the vulval precursor cells will express (Fig. 1*b*). The three cells P(5–7).p, which are near the anchor cell, are induced to generate the vulva: P6.p is determined to express a 1° lineage, whereas P5.p and P7.p are determined to express 2° lineages. The three cells P(3,4,8).p, which are farther from the anchor cell, are not induced and are determined to express a 3° lineage.

(3) Expression. During the middle to late L3 stage, the P(3–8).p cells express their lineages (1°, 2° or 3°); each lineage consists of a distinct pattern of cell divisions generating a particular set of cell types (Fig. 1*c*). The vulva is formed during the fourth larval (L4) stage by the descendants of P(5–7).p, which have expressed the 1° and 2° lineages.

Strategy for genetic analysis

To define a genetic pathway for the vulval cell lineages, we first observed the vulval cell lineages of individual hermaphrodites carrying an allele of each gene defined by a Muv or a Vul mutation. Description of the mutant phenotypes at the resolution of single cells allowed us to identify the first step in the developmental pathway visibly affected by a mutation in each gene. We confirmed these assignments by examining the patterns of epistasis among mutations postulated to affect different steps in the pathway. (One mutation is epistatic to a second mutation if the double mutant containing both mutations has the phenotype caused by the first mutation.) Because each step in the pathway of vulval development is dependent on the completion of the preceding step, a mutation that prevents the completion of an early step in the pathway should be epistatic to all mutations that prevent the completion of later steps. In addition, we determined the temperature-sensitive periods (TSPs) of those genes for which we had temperature-sensitive (ts) alleles. The TSP of a gene must be before, or concurrent with, all steps in the pathway for which that gene is needed.

Table 1 Lineages of P(3–8).p in mutants defective in the determination of precursor cell fates

Genotype	No. of animals	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p
Wild type		3°/S	3°	2°	1°	2°	3°
		S S*	S S	LLTN	TTTT	NTLL	S S
lin-10		3°/S	3°	3°	3°/1°	3°	3°
	8	S S*	S S	S S	S S	S S	S S
	3	S S*	S S	S S	TTTT	S S	S S
	2	S S*	S S	LLTN	TTTT	NTLL	S S
	1	S	S S	LLTN	S S	NTLL	S S
	1	S S	S S	S TN	S S	NT S	S S
lin-8; lin-9		2°/1°	1°/2°	2°/1°	1°/2°	2°	2°/1°
	1	LLOO	TTTO	LLLN	TTTO	NOLL	OTOO
	1	LOOT	LTTO	LLTN	TTTT	NLLL	LOTO
	1	LLOO	OOLL	LLLN	TTTT	NLLL	LLTO
	1	TTOO	LLLN	TTTT	NLLL	LLOO	LLO?
lin-13		3°/2°	2°/u	2°	1°	2°	3°/u
	1	LLOO	LLOO	LLON	T??T	NOLL	S S
	1	S S	S S	LLTN	TTTT	NLLL	S OO
	1	S	OODL	LLTN	O??T	NTLL	S S
	1	OLOT	S OO	LLTN	TTTT	NTLL	S S
	1	S S	S LL	LLON	TTTT	NTLL	TTLS

The lineages of the vulval precursor cells P(3–8).p in Vul hermaphrodites of genotype *lin-10(e1439)*, in Muv hermaphrodites of genotype *lin-8(n111); lin-9(n112)*, and in Muv hermaphrodites of genotype *lin-13(n387)*. In these strains, most of the aberrant lineages expressed by P(3–8).p were similar to one of the three lineages (1°, 2° or 3°) normally expressed by these cells in the wild type. Consequently, we have interpreted these lineage defects as transformations in the fates of the P(3–8).p cells. The top line for each gene shows a 'consensus fate' for each of the six cells; if a particular P(3–8).p cell expressed more than one fate, all fates observed in at least 25% of the animals examined are listed in the order of their frequencies and are separated by / . The subsequent lines for each gene describe the lineages of P(3–8).p in individual animals.

Lineage nomenclature is as follows. The lineage of each P(3–8).p cell is represented by one to four letters, each of which (with the exception of 'S'; see below) represents the fate of one of the four granddaughters of the P(3–8).p cell[8]. (Refer to Fig. 1 legend for the notation specifying cells.) The fate of each P(3–8).pxx cell comprises two parts: the axis of its division and the morphogenetic behaviours of its daughter cells. The axis of division is represented by individual letters (see Fig. 1 legend and below). The morphogenetic behaviour of the daughter cells is indicated by the style of the letter: if the daughters adhered to the ventral cuticle at the L3 molt, the letter is in boldface; if not, the letter is in roman typeface. We interpret the lineage of a P(3–8).p cell as a 1° lineage if all four P(3–8).pxx cells divided, represented by any of the letters 'T', 'O', 'L' or 'D' (see below), and if none of the eight descendent cells adhered to the ventral cuticle at the L3 molt, represented by roman typeface. We interpret the lineage of a P(3–8).p cell as a 2° lineage if at least three of the four P(3–8).pxx cells divided ('T', 'O', 'L' or 'D'), and if the four descendants of either P(3–8).pa or P(3–8).pp adhered to the ventral cuticle at the L3 molt, represented by two contiguous bold-face letters. We interpret the lineage of a P(3–8).p cell as a 3° lineage if neither of the P(3–8).px cells divided (a non-divided P(3–8).px cell is represented by 'S'). '1°', '2°', '3°', lineage with characteristics of the corresponding lineage of the wild type; 'u', uninterpretable lineage based on these criteria. Letters mean the following: 'N', 'T', 'L', as in the Fig. 1 legend; 'S', a P(3–8).p, P(3–8).px, or P(3–8).pxx cell appeared to join the hypodermal syncytium; 'O', a P(3–8).pxx cell divided with an oblique axis of division; 'D', a P(3–8).pxx cell divided, but its axis of division was not observed. '?', The fate of the P(3–8).pxx cell was not observed. Bold face indicates that the daughters of the P(3–8).pxx cell adhered to the ventral cuticle; otherwise, for 'T', 'L' and 'O', the daughters of the P(3–8).pxx cell moved dorsally within the hypodermis, and for 'N', the P(3–8).pxx cell moved dorsally within the hypodermis.

* In some animals P3.p did not divide and appeared to join the hypodermal syncytium.

Homoeotic defects in Muv and Vul mutants

We observed two general classes of vulval cell lineage defects in the Muv and Vul mutant strains. In strains of the first class, most of the mutant lineages expressed by the vulval precursor cells P(3–8).p are similar to one of the three wild-type lineages (1°, 2° or 3°) with regard to such criteria as time of cell divisions, axes of cell divisions and morphogenetic behaviour of descendent cells. We interpret such mutant lineages as transformations in the fates of the P(3–8).p cells. In Table 1, we present the vulval cell lineages of three such strains and describe the criteria used to classify the lineage of a particular P(3–8).p cell as a 1°, 2° or 3° lineage. In strains of the second class, most of the mutant lineages expressed by P(3–8).p are different from the normal 1°, 2° and 3° lineages. Nonetheless, many mutations of this second class also appear to cause transformations in the fates of the affected cells (see below).

In Table 2, we summarize the vulval cell lineages of Muv and Vul mutants defective in 22 genes. Most of these Muv and Vul mutations reduce or eliminate gene activity (refs 9, 11 and 12, summarized in Table 2), so the wild-type functions of most of these genes can be inferred from their mutant phenotypes.

Vulval cell lineages of mutants are variable

Unlike the vulval cell lineages of the wild type, those of the mutant strains we have studied are variable. The variability of the vulval cell lineages of some strains may result from residual gene activity (see Table 2), but such variability also occurs in strains in which we believe gene function has been eliminated. We have observed two types of variability. Some mutants have incompletely penetrant defects; for example in *lin-10* animals, P6.p expressed either the mutant fate (a 3° lineage) or the wild-type fate (a 1° lineage). We consider mutants of this class (mostly Vul mutants) to define genes that are involved in, and may be necessary for, the production of a particular cell fate or set of cell fates. In other mutants, a particular Pn.p cell can express one of several mutant fates; for example in *lin-8; lin-9* animals, P4.p, which expresses a 3° lineage in the wild type, expressed either a 1° lineage or a 2° lineage. (The Muv phenotype of *lin-8; lin-9* animals depends on both the *lin-8* and the *lin-9* mutations; see below.) We consider mutants of this class (mostly Muv mutants) to define genes that constrain a cell or set of cells to adopt a particular fate, so that if any such gene does not function, a cell may express any of a number of mutant fates.

We describe below how we have assigned 21 of the genes defined by Muv and Vul mutants, as well as two other genes involved in vulval development, to specific steps in the pathway of vulval development.

Genes for precursor cell generation

Mutations in five genes alter the fates of P(3–8).p before they divide in the L3 stage, indicating that these genes are involved in the generation of the vulval precursor cells. These five genes act at four temporally distinct steps. (1) Migration of P cell nuclei: mutations in *unc-83* and *unc-84* block the migrations of the nuclei of the P cells (the parents of P(3–8).p) into the ventral cord, preventing the subsequent development of the vulva[10,13]. (2) Generation of hypodermal Pn.p cells: the *lin-26* mutation causes most of the 12 Pn.p cells to have a neural nuclear morphology during the L1 stage and other Pn.p cells to divide during the L1 stage generating two, three, or less frequently four neural-like progeny (H. Ellis, personal communication), transforming the fate of the Pn.p cells to a fate possibly similar to that of their sister cells, the Pn.a neuroblasts. (3) Generation of hypodermal cells with the potential to become vulval precursor cells: in animals homozygous for the mutation *n300*, the cells P(3–8).p generally do not divide and appear to fuse with the hypodermal syncytium, adopting fates characteristic of their homologues P(1, 2, 9–11).p. (4) Expression of L3-specific cell lineages: in *lin-4* animals, the P(3–8).p lineages are highly variable with respect to both the timing of divisions and the number of descendent cells[14]. The descendants of these lineages do not have morphologies characteristic of vulval cells. Based on its effect on other cell lineages, the *lin-4* mutation seems to cause P(3–8).p, which normally would express the L3-specific vulval fates, to express instead a fate (generation of neural and hypodermal cells) characteristic of their parent P cells during the L1 stage[15].

To confirm that the genes involved in the generation of the vulval precursor cells act before genes involved in later steps of vulval development, we have examined the phenotypes of 33

Table 2 Genes that affect the vulval cell lineages

Gene	Allele(s) used	Phenotype	Nature of phenotype; effect on gene activity	Cell fates P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	P(1, 2, 9–11).p	No.
Wild type										3°/S	3°
Generation mutants											
lin-26	*n156*	Vul	Recessive; reduction	n	n	n	n	n	n	n	9
—*	*n300*	Vul	Recessive; reduction or elimination	S	S	S	S	S	S	S	15
lin-4	*e912*	Vul	Recessive; reduction or elimination	S/r	r	r	r	r	r	S	3
Determination mutants											
lin-2	*e1309* *e1453*	Vul	Recessive; probably elimination	3°/S	3°	3°	3°/u	3°	3°	S	9
lin-3	*e1417*	Vul	Recessive; reduction	3°/S	3°	3°	3°/1°	3°	3°	S	12
lin-7	*e1413*	Vul	Recessive; elimination	3°/S	3°	3°/2°/u	3°/1°	3°/u	3°	S	7
lin-10	*e1439*	Vul	Recessive; elimination	3°/S	3°	3°	3°/1°	3°	3°	S	15
let-23	*n1045*	Vul	Recessive; reduction	3°/S	3°	3°	3°	3°	3°	S	41
lin-1	*e1777* *e1275*	Muv	Recessive; elimination	1°/u	2°	1°/2°	1°	2°	u/1°	S	6
lin-17	*n671* *n677*	Muv	Recessive; reduction or elimination	3°/S	3°	2°	1°	ab2°/2°	3°	S	9
lin-18	*e620*	Muv	Recessive; elimination	3°/S	3°	2°	1°	ab2°/2°	3°	S	13
lin-15	*n309*	Muv	Recessive; reduction	2°	2°/1°	2°	1°	2°	2°/1°	S	5
lin-13	*n387*	Muv	Recessive; reduction	3°/2°	2°/u	2°	1°	2°	3°/u	S	5
lin-8(n111); lin-9(n112)		Muv	Recessive; reduction or elimination†	2°/1°	1°/2°	2°/1°	1°/2°	2°	2°/1°	S	4
lin-12(0)	*n137/ n720*	Abn	Recessive; elimination	S/3°	u/3°	1°	1°	1°/u	3°	S	11
lin-12(d)	*n137*	Muv	Dominant; increased or ectopic expression	2°	2°	2°	2°	2°	2°	S	11
Expression mutants											
lin-11	*n382* *n389*	Vul	Recessive; reduction or elimination	S/3°	3°	ab2°	1°	ab2°	3°	S	7
Other mutants											
lin-24	*n432*	Vul	Dominant; novel function?	X/3°	X/3°	X/3°	X/1°	X/3°	X/3°	X/S	22
lin-33	*n1043*	Vul	Dominant; novel function?	X/3°	X/3°	X/3°	X/1°	X/3°	X/3°	X/S	15
lin-34	*n1046*	Muv	Dominant; unknown	(1°–2°)/3°	(1°–2°)	2°	1°	2°	(1°–2°)/3°	S	12
lin-31	*n301*	Muv	Recessive; elimination	d, (u/2°)	d, (u/2°)	d, (2°/u)	d, (1°/u)	d, (2°/1°)	d, (2°/3°)	S	11, 4
lin-25	*e1446*	Vul	Recessive; reduction or elimination	d, (3°)	d, (3°)	d, (3°)	d, (1°/u)	d, (3°)	d, (3°)	d/S	14, 5

The alleles listed are those used for the determination of the vulval cell lineages and/or for studies of gene interactions. The effects of Muv and Vul mutations on the activities of their respective genes are based on genetic analyses[9,11,12]. The fates of P(1–11).p in animals of all genotypes except those listed below were determined using Nomarski optics to observe directly the divisions and patterns of differentiation of each of these cells and their descendants. The fates of P(1–11).p in *lin-34* animals, *let-23* animals, *lin-33* animals, some *lin-31* animals, and some *lin-24* animals were inferred from observations using Nomarski optics after the vulval cell divisions were complete. Animals of genotype *let-23* were grown at 15 °C before observation. The vulval cell lineages of *lin-4* animals are from ref. 14. For most strains, we examined the vulval anatomy of additional animals to confirm that the above lineages were representative of the defects in the strain. For each genotype, the consensus fates of P(1–11).p are described as in Table 1. In those strains, *lin-25* and *lin-31*, in which some of the cells P(1–11).p divided before the L3 stage, indicated by 'd' the fates shown in parentheses are those the P(1–11).p cell or its descendants adopted during the L3 stage. 'Generation' mutants define genes necessary for the generation of P(3–8).p cells with the potential to express vulval cell lineages (see text). 'Determination' mutants define genes necessary for the proper determination of the fates of the P(3–8).p cells in response to the inductive signal from the anchor cell. In such mutants, some of the P(3–8).p cells express 1°, 2°, or 3° lineages that are normally expressed only by others of these cells. The criteria used to distinguish 1°, 2°, and 3° lineages are described in the legend to Table 1. For general descriptions of the vulval cell lineages of animals of genotypes *lin-1*, *lin-2*, *lin-3*, *lin-7* and *lin-8; lin-9*, see ref. 10. The vulval cell lineages of animals of genotypes *lin-12(d)*, *lin-12(0)*, *lin-15*, *let-23* and additional animals of genotypes *lin-2*, *lin-3* and *lin-7* will be presented elsewhere (P.W.S. and H.R.H., in preparation). 'Expression' mutants define genes necessary for the expression of one of the three vulval fates (see text). We were unable to assign 'Other' mutants to specific steps in the pathway of vulval development (see text). For *lin-25* and *lin-31*, the first number is the number of animals observed during the L1 or L2 stage to confirm the presence of Pn.p divisions before the L3 stage, and the second number is the number of animals for which vulval cell lineages were observed during the L3 stage. 'Muv', multivulva; 'Vul', vulvaless; 'Abn', abnormal vulva. Descriptions: '1°', '2°', '3°', 'u', as in Table 1; other entries as follows: '(1°–2°)', by anatomical criteria expressed a 1°-like or 2°-like lineage, but the lineage was not directly observed; 'ab2°', underwent an abnormal 2° lineage (see text); 'S', did not divide and appeared to join the hypodermal syncytium; 'd', divided precociously; 'n', the nucleus had a compact neural appearance or generated progeny with compact neural nuclei; 'X', had abnormal morphology in the L1 stage and sometimes died; 'r', divided variably, possible reiteration of ancestral fate (see text).

*—Here *n300* refers to the mutation associated with the Vul phenotype observed in animals homozygous for the reciprocal translocation *nT1(IV;V)*[12]. Because no single gene mutation has been isolated that fails to complement the Vul phenotype caused by *n300*, no gene name has been given to this mutation.

†—The Muv phenotype of *lin-8; lin-9* animals is dependent on the presence of both the *lin-8* and the *lin-9* mutations, each of which alone confers a wild-type phenotype (ref. 9; also, we examined at the level of single cells the vulval anatomy of ~20 animals of each of the two genotypes; all *lin-8* and all *lin-9* animals were wild-type).

Table 3 Gene interactions

	Determination Muv				
	lin-1	*lin-12* (*n137*)	*lin-15*	*lin-8; lin-9*	*lin-13*
Generation Vul					
lin-26 *n300* *lin-4* *lin-24(n432)*	Vul*	Vul	Vul	Vul	Vul
Determination Vul					
let-23	Muv	Muv	Muv/Vul	Vul	Vul
lin-2 *lin-7* *lin-10*	Muv	Muv	Muv	Muv/Vul	Vul
lin-3	Muv	Muv	Muv	Muv	Muv

The interaction between a multivulva and a vulvaless mutation was examined using a dissecting microscope to determine the vulval anatomy of a strain that contained both mutations and to compare the gross phenotype of the double mutant to that of each single mutant, which has been described[12]. Double mutant strains were constructed by picking ~10 homozygous Muv and 10 homozygous Vul animals from the progeny of doubly heterozygous hermaphrodites. As some of the Muv or Vul animals that were picked were also heterozygous for a mutation of the opposite phenotype (for example, some homozygous Muv animals were heterozygous for the Vul mutation), the double mutant strain was obtained in the next generation by picking a segregant of a Muv or Vul animal that had a phenotype different from that of its parent (such as a Vul animal derived from a Muv parent). The Muv and Vul alleles used are listed in Table 2: *lin-1(e1275)* was used to construct most double mutants containing *lin-1*, and *lin-2(e1309)* was used to construct most double mutants containing *lin-2*. In a few double mutants, alternative alleles were used; *lin-2(e1453)* was used for the *lin-12(d); lin-2* and *lin-2 lin-15* strains; *lin-10(e1438)* was used for the *lin-10; lin-8; lin-9* strain; and *lin-1(e1777)* was used for the *let-23; lin-1* strain. Other than those listed, additional double mutants containing a mutation that affected the generation of the vulval precursor cells and a mutation that affected a later step in the pathway of vulval development were also constructed. In all cases the mutation that affected the generation of the vulval precursor cells was epistatic to the mutation that affected the later step. Specifically, *unc-84(e1410)* was epistatic to mutations in genes affecting the determination of the fates of the precursor cells—*let-23, lin-12(d)*, and *lin-8; lin-9*—as well as to mutations in genes affecting the expression of the vulval lineages—*lin-11* and *lin-18*. In addition, the vulval cell lineage defects resulting from *n300* were present in double mutants carrying *n300* and Vul mutations in any of three genes—*lin-2, lin-7*, and *lin-10*—that affect the determination of the fates of the precursor cells, and the vulval cell lineage defects resulting from *lin-24* were present in *lin-24; lin-2* and *lin-10; lin-24* double mutants. Lastly, *lin-26, n300, lin-4*, and *lin-24* were epistatic to the Muv mutation *lin-31*. Muv, the double mutant strain had a Muv phenotype; Vul, the double mutant strain had a Vul phenotype; Muv/Vul, the phenotypes resulting from the Muv and Vul mutations were co-expressed in the double mutant strain. In Muv/Vul strains, some animals had Muv characteristics, some were Vul, and some animals had both Muv and Vul characteristics. We examined animals of some such strains using Nomarski optics; in certain animals of each strain examined, some of the cells P(3,4,8).p, which express a 3° lineage in the wild type, expressed 1° or 2° lineages, while some of the cells P(5–7).p, which express a 1° or 2° lineage in the wild type, expressed 3° lineages.

* As the translocation *nT1(IV;V)* suppresses recombination in an interval of LGIV that includes *lin-1*[12], the double mutant containing *lin-1* and the mutation causing the Vul phenotype associated with *nT1*, *n300*, was not constructed.

strains carrying a mutation affecting the generation of the vulval precursor cells and a mutation postulated to affect a later step. In all of these strains, the mutation affecting the generation of the vulval precursor cells was epistatic to the second mutation in the strain (Table 3). For example, the Vul mutation *n300*, which causes the vulval precursor cells to join the hypodermal syncytium without dividing, is epistatic to all Muv mutations that cause the six vulval precursor cells to express 1° and 2° vulval lineages. Thus, *n300* disrupts a step necessary for the generation of cells capable of expressing vulval lineages.

A gene controlling anchor cell generation

Greenwald *et al.*[11] have shown that the gene *lin-12* controls the generation of the anchor cell. Recessive *lin-12(0)* loss-of-function mutations cause both potential anchor cells (Z1.ppp and Z4.aaa in Fig. 1*a*) to become anchor cells. Conversely, dominant *lin-12(d)* elevation-of-function mutations cause both of these cells to become VU cells. Thus, the level of *lin-12* activity specifies whether a potential anchor cell will become an anchor cell or a VU cell. (Abnormalities in anchor cell number caused by *lin-12* mutations have an effect on the fates of the vulval precursor cells distinct from the direct action of *lin-12* in specifying the fates of these cells; see below and P.W.S. and H.R.H., in preparation.)

Genes for precursor cell determination

We have identified 15 genes that affect the determination of which of the three alternative vulval lineages each precursor cell expresses. Reduction-of-function mutations in any of these genes cause some precursor cells to adopt fates characteristic of other precursor cells. Three classes of transformations in cell fates are known (Table 1, ref. 10; P.W.S. and H.R.H., in preparation; summarized in Table 2). (1) In Vul mutants defective in *lin-2, lin-3, lin-7, lin-10* or *let-23*, the P(5–7).p cells generally express 3° lineages. Thus, these genes are necessary for the generation of the 1° and 2° lineages (see Fig. 1*c* for a description of each lineage). (2) In Muv mutants defective in *lin-1, lin-15* or *lin-13*, P(3,4,8).p often express 1° or 2° lineages. Animals of genotype *lin-8; lin-9* have a similar Muv phenotype. However, the Muv defect of this strain is dependent upon two mutations: either the *lin-8* or the *lin-9* mutation alone confers a wild-type phenotype (ref. 9 and Table 2 legend). In addition, mutations in any of four other genes—*lin-35, lin-36, lin-37* and *lin-38*—result in a wild-type phenotype on their own but generate a Muv phenotype with a *lin-8* or a *lin-9* mutation (E.L.F. and H.R.H., in preparation). Thus, we have identified nine genes with activities that, either singly or in certain pairwise combinations, appear necessary for the generation of the 3° lineage. These genes may act by promoting the 3° lineage and/or by repressing the 1° and 2° lineages. (3) Recessive loss-of-function *lin-12(0)* mutations cause P(3–8).p generally to express 1° or 3° lineages, but never 2° lineages[11]. Conversely, dominant elevation-of-function *lin-12(d)* mutations, which result in a Muv phenotype, cause P(3–8).p to express 2° lineages[11]. Thus, the level of *lin-12* activity specifies whether a P(3–8).p cell will express a 2° lineage or a non-2° lineage.

Site of action of precursor cell determination genes. The determination of the fates of the vulval precursor cells involves the production of an inductive signal by the gonadal anchor cell and the response to it by the hypodermal vulval precursor cells. Thus, mutants abnormal in the determination of precursor cell fates could be defective either in the gonad or in the hypodermis. For example, the expression of 1° and 2° lineages by P(3,4,8).p in the Muv mutants could be caused by (1) a defect in the gonad resulting in an increase in the strength of the inductive signal, (2) a defect in the hypodermis resulting in an increased sensitivity to the inductive signal, or (3) a defect in the hypodermis resulting in the expression of 1° and 2° lineages independently of an inductive signal. To determine whether any of the Muv mutations are of the third class, we have used a laser microbeam[5,16] to ablate the gonads of five L1 animals of each of the Muv genotypes *lin-1, lin-12(d), lin-13, lin-15*, and *lin-8; lin-9*. (The ablation of the gonad of an L1 wild-type animal causes all six vulval precursor cells to assume a 3° fate[5].) All 25 mutant animals became Muv, indicating that the expression of 1° and 2° lineages in these strains is independent of the gonadal inductive signal. Thus, the site of action of these signal-independent Muv mutations seems likely to be within the hypodermal vulval

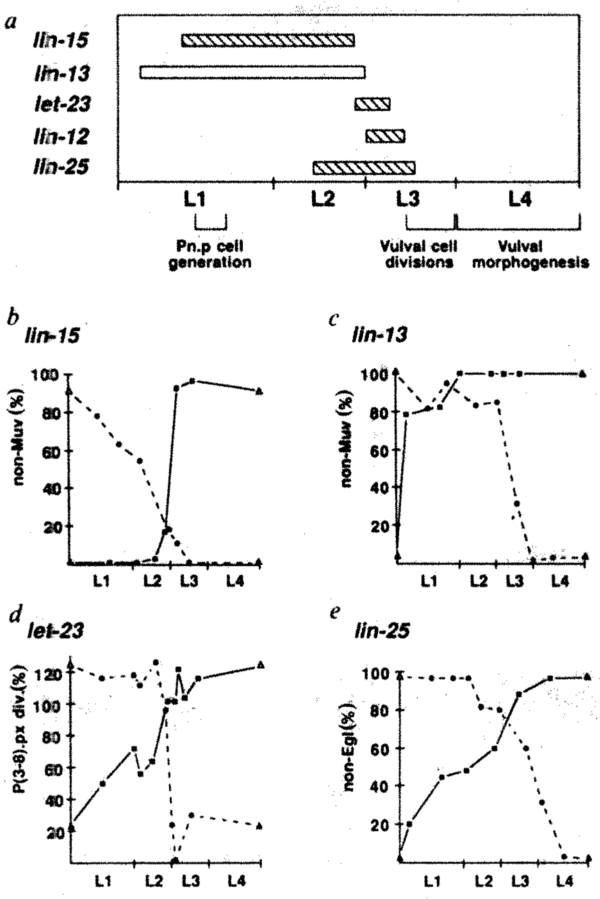

Fig. 2 Temperature-shift experiments. *a*, Temperature-sensitive periods (TSPs) (hatched rectangles) for *lin-15(n765)*, *let-23(n1045)*, *lin-12(n137 n460)*, and *lin-25(n545)* activities; these TSPs are the minimal periods based on our data during which the gene product must be active to generate a wild-type phenotype. The open rectangle indicates the period, which is not strictly a TSP (see below), during which *lin-13(n387)* animals must be at the restrictive temperature to display an essentially mutant phenotype. The beginning of the TSP is defined[24] as the earliest point at which a shift from the restrictive to the permissive temperature results in a higher percentage of mutant animals than that observed in animals maintained at the permissive temperature. Similarly, the end of the TSP is defined[24] as the latest point at which a shift from the permissive temperature to the restrictive temperature results in a higher percentage of mutant animals than that observed in animals maintained at the permissive temperature. The TSP of each gene could encompass a slightly larger period of time than shown in the diagram, in that the beginning of each TSP could extend up to, but not include, one point earlier than indicated and the end could extend up to, but not include, one point later than indicated. We have chosen not to present this larger interval because we have no data that indicate that these genes act beyond the periods shown. The TSP of *lin-12(n137 n460)* is based on ref. 11. Panels *b–e*: (■ and solid lines), percentage of animals shifted from the permissive to the restrictive temperature at the stage indicated that expressed a non-mutant phenotype; (● and dotted lines), percentage of animals shifted from the restrictive to the permissive temperature at the stage indicated that expressed a non-mutant phenotype. △, Percentage of animals grown at the permissive temperature that expressed a non-mutant phenotype. ▲, For *lin-13*, *lin-15* and *lin-25*, the percentage of animals shifted from the permissive to the restrictive temperature before hatching that expressed a non-mutant phenotype; for *let-23*, the percentage of animals grown at the restrictive temperature that expressed a non-mutant phenotype.

Methods. In *b*, stocks of *lin-15(n765)* animals were grown at the permissive temperature of 15 °C. A synchronous population was obtained by placing three or four adult hermaphrodites on each of 36 Petri dishes equilibrated at 15 °C and allowing these animals to lay eggs for two hours. Immediately after removal of the parents, half the Petri dishes were shifted to the restrictive temperature of 25 °C and half were kept at 15 °C. At various later times, two Petri dishes, containing between 23 and 86 animals, were shifted either from 15 °C to 25 °C or from 25 °C to 15 °C. In most cases, the stage of development of three or four of the animals that were shifted was ascertained using Nomarski optics[1]; otherwise, the stage of the animals at the time of transfer was inferred from animals observed at other time points during the same experiment. Two Petri dishes at each temperature were not shifted. 'non-Muv (%)', percentage of *lin-15* animals that did not have multiple pseudovulvae after reaching adulthood. (The wild type is 100% non-Muv.) In *c*, because *lin-13(n387)* results in a sterile Muv phenotype, hermaphrodites of genotype *+lin-13 unc-36(e251)+/lin-16(e1743)++unc-86(e1507)* were grown at the permissive temperature of 15 °C. The temperature-shift experiments were performed on the progeny of these animals as in '*b*'. The temperature-shift data indicate that most (>75%) *lin-13* animals have essentially wild-type phenotypes if they are grown at the permissive temperature for any period of time between the early L1 stage and the L2 molt. This 'temperature-sufficient period' of *lin-13* could result from either of two causes[25]. First, the *lin-13* mutation could be temperature-sensitive for the synthesis of its gene product, with the product made in excess continuously from the early L1 until the L2 molt, after which it functions. Alternatively, *lin-13* could have two TSPs, one ending at the beginning of the temperature-sufficient period (the early L1) and the other starting after that time and ending at the end of the temperature-sufficient period (the L2 molt), with *lin-13(+)* activity during either of the TSPs sufficient to generate a wild-type phenotype. The latter hypothesis is consistent with the observation[12] that at the permissive temperature *lin-13(+)* acts both maternally and zygotically. 'non-Muv (%)', as in *b*. In *d*, stocks of *let-23(n1045)* animals were grown at the permissive temperature of 25 °C and at the restrictive temperature of 15 °C. Individual animals were shifted from 25 °C to 15 °C or from 15 °C to 25 °C. The stage of development of each animal was ascertained using Nomarski optics at the time of the shift[1,6]. Between 1 and 15 animals were shifted at each time point. 'P(3–8).px div. (%).', the number of P(3–8).px cells that divided in individual animals compared to the six (P(5–7).px) that divide in the wild type. (The wild type is 100% P(3–8).px div.) At the permissive temperature, *let-23(n1045)* causes more P(3–8).px cells to divide than in the wild type; this phenotype, the basis of which will be discussed elsewhere, is likely to result from a small decrease in *let-23* gene activity (P.W.S. and E.L.F., unpublished observations.) In *e*, stocks of *lin-25(n545)* animals were grown at the permissive temperature of 15 °C and temperature-shift experiments were performed as in *b*. 'non-Egl (%)', percentage of adult *lin-25* animals competent to lay eggs. (The wild type is 100% non-Egl.) As hermaphrodites with certain vulval cell lineage defects nonetheless can lay eggs (ref. 10 and P.W.S., unpublished observations), the egg-laying competent *lin-25* animals may not have expressed entirely wild-type vulval lineages.

precursor cells.

Similarly in Vul mutants, P(5–7).p could adopt 3° lineages either because the activity of the inductive signal of the gonad is reduced or because the ability of the precursor cells to respond to the inductive signal is reduced. By examining the phenotypes of double mutants carrying a Vul mutation and any of the signal-independent Muv mutations described above, we can distinguish certain classes of Vul mutations. Just as the physical ablation of the gonad does not affect the Muv phenotype of these mutants, the 'genetic ablation' of the gonadal signal by a gonad-specific Vul mutation should not affect the phenotype caused by a signal-independent Muv mutation. Conversely, if a Vul mutation has a site of action in the hypodermis, the phenotype of the Vul mutation could be epistatic to, co-expressed with (see Table 3 legend), or not alter the phenotypic effect of a signal-independent Muv mutation. Thus, if a Vul mutation affects the Muv phenotype caused by any signal-independent Muv mutation, the Vul mutation must have a site of action outside the gonad and thus presumably in the hypodermis. However, if all signal-independent Muv mutations are epistatic to a Vul mutation, the site of action of the Vul mutation could be either in the gonad or in the hypodermis.

Vul mutations in *let-23*, *lin-2*, *lin-7* and *lin-10* affected the Muv phenotypes of at least some hypodermal-acting Muv mutations (Table 3), indicating that mutations in these four genes have sites of action in the hypodermal precursor cells. The site of action of *lin-3* was not revealed by these experiments (all signal-independent Muv mutations were epistatic to the Vul allele *lin-3(e1417)*), but the Vul phenotype resulting from a further reduction in *lin-3* activity was co-expressed with the signal-independent Muv phenotype resulting from a less severe *lin-15* allele, indicating that *lin-3* also affects the hypodermis. Specifically, at 20 °C the Muv phenotype of animals of genotype *lin-3(n378)/lin-3(n1059); lin-15(n765ts)* is less severe than the Muv phenotype of *lin-15(n765ts)* animals. (Note that *n378* is a Vul allele of *lin-3*, *n1059* is a putative null allele of *lin-3* and results in larval lethality, and *n765ts* is a *lin-15* allele that at 20 °C results in a less expressive Muv phenotype than the reference allele of *lin-15*, *n309*; ref. 12.)

Thus, all mutations that affect the determination of the fates of the precursor cells appear to have a site of action in these cells, implying that these gene products are involved in the reception of and/or response to the inductive signal. We have failed to identify any genes in which loss-of-function mutations

Table 4 Lineages of P(5–7).p in mutants defective in expression of 2° lineages

Genotype	No. of animals	P5.p	P6.p	P7.p
Wild type		2°	1°	2°
		LLTN	TTTT	NTLL
lin-11		ab2°	1°	ab2°
	1	LLLL	TOOT	LLOO
	1	LLLO	TTOT	OLOL
	1	LLLL	OTTO	OOLL
	1	LLL?	TTTT	?LLO
	1	LLLO	TTOT	OOLL
	1	LOLO	TTOT	OLLL
	1	OLLO	TTTT	LLOL
lin-17		2°	1°	ab2°
	4	LLTN	TTTT	LLLL
	1	LLTN	TTTT	LLTN
	1	LLTT	T?TT	LLLL
	1	LLLT	TTTT	LLTT
	1	LLTN	TTTT	TTLL
	1	LLTN	TTTT	LLOO
	1	LLTT	TTTT	LLLL
	1	LLTT	TTTT	LLLO
	1	LLTT	TTTT	TOLL
lin-18		2°	1°	ab2°/2°
	3	LLTN	TTTT	NTLL
	2	LLTN	TTTT	LLNT
	1	LLTN	TTTT	TTLL
	1	LLTN	TTTT	LLTN
	1	LLTN	TTTT	LLNO
	1	LLTN	TTTT	LLLL
	1	LLTN	TTTT	TOLL
	1	LLLL	TDTT	LLLL
	1	LLTO	TTTT	LLLL
	1	LLTT	TTTT	NTLL

The lineages of P(5–7).p in *lin-11(n382, n389)* hermaphrodites, *lin-17(n671, n677)* hermaphrodites and *lin-18(e620)* hermaphrodites are represented using the nomenclature of Table 1 legend. (In all animals of these genotypes, the other three precursor cells P(3,4,8).p underwent lineages identical to those expressed by the equivalent cells in the wild type.) In these strains, the lineages of P5.p and P7.p, the cells determined to express a 2° lineage in the wild type, can be abnormal. The top line of each entry summarizes the 'consensus fates' expressed by P(5–7).p in each strain (see the legend to Table 1). The subsequent lines of each entry contain the lineages of P(5–7).p in individual animals of each strain. For *lin-11*, the first four lines represent lineages of *lin-11(n382)* animals, and the remaining three lines represent lineages of *lin-11(n389)* animals. In *lin-11* animals, all four P5.p and P7.p daughter cells express an [LL]-like pattern of division. (Although some P5.pxx or P7.pxx cells in *lin-11* animals divided with an oblique axis, [O], their descendants adhered to the ventral cuticle, as do the descendants of an [L] lineage.) For *lin-17*, the first six lines represent lineages of *lin-17(n671)* animals, and the remaining three lines represent lineages of *lin-17(n677)* animals. Note that *lin-18(e620)* results in a slightly temperature-sensitive phenotype. For *lin-18*, the first five lines represent lineages of *lin-18* animals grown at 20 °C. The remaining five lines represent lineages of *lin-18* animals that were grown at 25 °C until the P(5–7).pxx cells had divided. These animals were then placed at 20 °C and the final round of cell divisions was observed; cell lineages were inferred based upon the positions of the P(3–8).pxx cells in individual animals. In some *lin-17* and *lin-18* animals, the lineage of P7.p was symmetric but the fates expressed by all four P7.pxx cells were abnormal (the P7.pxx cells divided with a longitudinal axis, but their daughter cells did not adhere to the ventral cuticle, represented by [LLLL]). Descriptions: '1°', '2°', 'L', 'T' 'N', 'O', 'D', '?', as in Table 1 legend; 'ab2°', expressed abnormal 2° lineages with a defect characteristic of the particular mutant strain (see text).

eliminate the inductive signal of the anchor cell. Perhaps the genes that function in the production of the signal have essential functions and a loss-of-function mutation in any such gene results in lethality. Alternatively, it may not be possible to eliminate the inductive signal by a mutation in a single gene, either because the genes that control the signal are multigene family members or because the signal itself has multiple at least partially redundant components. It also remains possible that any of the genes classified as involved in the response to the signal could also affect its production. In our experiments, any mutation that caused a defect in both the production of and the response to the signal would be scored simply as defective in the response to the signal. Moreover, one or more of the genes defined by Vul mutations may normally act in the gonad but appear to be hypodermal-acting by our tests; in particular, a mutation might result in a Muv phenotype by causing such a gene to be active in the hypodermis, in which case the Vul mutation would be epistatic to the Muv mutation (because in the Muv mutant background the gene defined by the Vul mutation would indeed be acting in the hypodermis).

Times of action of presursor cell determination genes. We have performed temperature-shift experiments using temperature-sensitive alleles of three genes we believe to be involved in the determination of the precursor cell fates—heat-sensitive alleles of *lin-13* and *lin-15* and a cold-sensitive allele of *let-23*. The results are consistent with the hypothesis that these genes are involved in the determination of the fates of the precursor cells as opposed to the expression of those fates (Fig. 2). Specifically, the period during which the temperature-sensitive activity of each gene is needed to generate a wild-type phenotype ends before the onset of vulval cell divisions in the middle of the L3 stage. If the TSP of any of these genes had extended beyond the onset of vulval cell divisions (as is the case for *lin-25*; see below), we would have concluded that that gene acts after the period in which vulval precursor cell fates are determined.

Our temperature-shift data also suggest that these three genes, as well as *lin-12*[11], act during different periods of larval development. Specifically, *lin-15* and possibly *lin-13* appear to function from the L1 stage until the late L2 stage, *let-23* appears to function from the late L2 stage to the early L3 stage and *lin-12* appears to function during the early L3 stage. However, if the TSPs of any of these genes reflect temperature-sensitive defects in the synthesis of gene products (Fig. 2, legend), the results of these experiments simply indicate that these gene products are synthesized before the onset of vulval cell divisions and may function at any time after these divisions.

Genes for 2° lineage expression

We have identified three genes that are necessary for the expression of the 2° lineages. In the wild type, the two vulval precursor cells that are determined to undergo a 2° lineage, P5.p and P7.p, express asymmetric lineages of opposite polarity: P5.p undergoes a lineage represented as [LLTN], and P7.p undergoes a mirror-symmetric lineage represented at [NTLL]. (Each letter represents the fate of a P(3–8).pxx cell, one of the granddaughters of the P(3–8).p cell; see the legend to Fig. 1, and ref. 8 for details.) The first division of the 2° lineage is asymmetric, as the two cells generated express different fates, [LL] and [TN].

In mutants defective in *lin-11*, *lin-17* or *lin-18*, the asymmetry of the 2° lineages is disrupted (Table 4). In *lin-11* mutants, the 2° lineages are symmetric; all four P5.p and P7.p daughter cells express the [LL] portion of the 2° lineage, so that many P5.p and P7.p cells undergo [LLLL] lineages. In *lin-17* and *lin-18* mutants, the asymmetry of the P7.p lineage can be disrupted in two ways. In some *lin-17* and *lin-18* animals, the lineage expressed by P7.p is symmetric, but each P7.px cell expresses a novel fate [**LL**]. (The fate [**LL**] indicated with bold type is distinct from the fate [LL] indicated in non-bold type; see Table 4, legend.) In other animals of these genotypes, the polarity of the P7.p lineage is reversed, so that P7.pa rather than its sister

Table 5 Specificity for 2° lineages exhibited by expression mutants

	Wild type						*lin-11, lin-17* or *lin-18*					
	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p
Wild type	3°/S	3°	2°	1°	2°	3°	3°/S	3°	2°/ab2°	1°	ab2°/2°	3°
lin-12(d)	2°	2°	2°	2°	2°	2°	ab2°/2°	ab2°/2°	ab2°/2°	ab2°/2°	ab2°/2°	ab2°/2°
lin-12(0)	S/3°	u/3°	1°	1°	1°/u	3°	3°/S	3°/u	1°	1°	1°/u	3°
ac⁻	3°/S	3°	3°	3°	3°	3°	3°/S	3°	3°	3°	3°	3°

The expression of the *lin-11, lin-17* and *lin-18* phenotypes in animals in which all, some, or none of the P(3–8).p cells are determined to express a 2° lineage. In *lin-12(d)* animals, the 2° lineage of each of the vulval precursor cells has a characteristic polarity (P.W.S. and H.R.H., in preparation): P(3–5).p express 2° lineages with the polarity of the wild-type P5.p lineage, P6.p expresses a 2° lineage with the polarity of the wild-type P7.p lineage, and P7.p and P8.p can express a 2° lineage of either polarity. In *lin-11; lin-12(d)* hermaphrodites (6 animals), all six precursor cells expressed [LLLL]-like lineages. In *lin-17; lin-12(d)* hermaphrodites (5 animals) and *lin-12(d); lin-18* hermaphrodites (2 animals), all six precursor cells either expressed a 2° lineage with the polarity of P5.p or expressed a symmetrical but abnormal lineage [LLLL], which is characteristic of *lin-17* and *lin-18* animals (see text). In *lin-12(0)* animals, none of the six precursor cells is determined to express a 2° lineage. In *lin-11; lin-12(0)* hermaphrodites (10 animals), *lin-17; lin-12(0)* hermaphrodites (5 animals), or *lin-12(0); lin-18* hermaphrodites (4 animals), none of the six precursor cells expressed any of the defects associated with *lin-11, lin-17* or *lin-18*. (The vulval cell lineages of *lin-11; lin-12(0)* animals were not determined directly; instead, these animals were found to be similar in vulval anatomy to *lin-12(0)* animals, indicating that the *lin-11* defect probably is not expressed in a *lin-12(0)* animal.) *lin-11, lin-17* and *lin-18* animals that lacked an anchor cell were obtained by ablating the gonads of seven *lin-11*, five *lin-17*, and seven *lin-18* L1 animals with a laser microbeam. Descriptions '1°', '2°', '3°', 'u', 'S', as in the legend to Table 1; 'ab2°', expressed an abnormal 2° lineage characteristic of *lin-11, lin-17*, or *lin-18*.

cell P7.pp undergoes an [LL] lineage. Note that *lin-17* mutations also cause at least five other blast cells that normally undergo asymmetric cell divisions to produce daughter cells with identical, although sometimes abnormal, fates (P.W.S. and H.R.H., in preparation). Thus, it is likely that mutations in *lin-11*, as well as mutations in *lin-17* and possibly *lin-18*, cause certain sister cells that normally adopt different fates to adopt similar fates instead.

If these three genes specifically affect the expression of the 2° lineages, the defects caused by mutations in these genes should be observed in all vulval precursor cells that are determined to express a 2° lineage and should not be observed in any vulval precursor cells determined to express a 1° or a 3° lineage. We tested this hypothesis by observing the expression of the *lin-11, lin-17* and *lin-18* defects in animals in which either all or none of the six precursor cells were determined to express 2° lineages (Table 5). First, a *lin-12(d)* mutation causes all P(3–8).p cells to be determined to express a 2° lineage (see above); in double mutants carrying a *lin-12(d)* mutation and a *lin-11, lin-17* or *lin-18* mutation, all six P(3–8).p cells displayed the defects associated with a *lin-11, lin-17* or *lin-18* mutation. Second, a *lin-12(0)* mutation causes all P(3–8).p cells to be determined to express non-2° lineages; in double mutants carrying a *lin-12(0)* mutation and a *lin-11, lin-17* or *lin-18* mutation, none of the six P(3–8).p cells displayed any of the defects associated with a *lin-11, lin-17* or *lin-18* mutation. Third, ablation of the gonad in L1 wild-type animals causes all P(3–8).p cells to be determined to express 3° lineages; in gonad-ablated *lin-11, lin-17* and *lin-18* animals, all six P(3–8).p cells expressed a 3° fate.

Thus, *lin-11, lin-17* and *lin-18* appear to be required specifically for the expression of the 2° lineage. Furthermore, the determination of the fates of the vulval precursor cells and the expression of the vulval lineages (or at least the expression of the 2° lineage) are independent, genetically separable steps.

Genes not assigned to specific steps

We have been unable to assign five genes to specific steps in the pathway of vulval development. Three of these genes—*lin-24, lin-33* and *lin-34*—are defined by partially dominant mutations that cause defects in particular steps in the pathway. However,

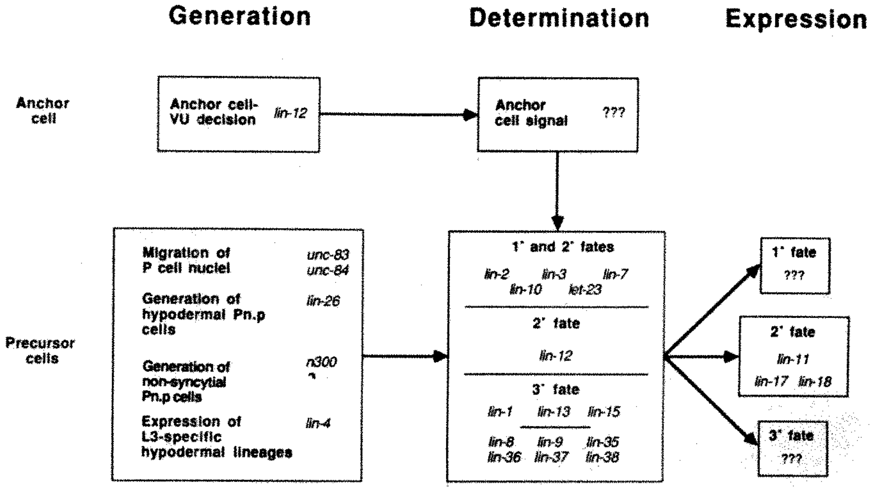

Fig. 3 A genetic pathway for the specification of the vulval cell lineages (see text). The postulated order of the five genes involved in the generation of the vulval precursor cells is indicated from top to bottom within the box. This order is based only upon mutant phenotypes; epistasis experiments have not been performed. Mutations in two other genes besides *lin-4* cause the vulval precursor cells to adopt fates normally expressed at other times during development[15]. Dominant mutations in *lin-14* cause a Vul phenotype identical to that observed in *lin-4* animals; recessive mutations in *lin-14* or *lin-28* cause P(3–8).p to express vulval-like cell lineages during the L2 stage. However, as the activities of *lin-14* and *lin-28* are not necessary for the expression of vulval cell lineages, we have omitted these genes here. The nine genes necessary for the expression of the 3° lineage are divided into two classes. The genes *lin-1, lin-13* and *lin-15* have been identified by mutations that result in a Muv phenotype; *lin-8, lin-9, lin-35, lin-36, lin-37* and *lin-38* have been identified by mutations that alone result in a wild-type phenotype but in certain pairwise combinations result in a Muv phenotype (see text). The gene *lin-12* acts twice in the pathway. First, *lin-12* is involved in the specification of the fates of the two bipotent cells, Z1.ppp and Z4.aaa, one of which becomes the anchor cell. Second, *lin-12* activity is necessary for the vulval precursor cells to adopt 2° lineages.

there are no known loss-of-function alleles of these genes[12]. Because dominant mutations can cause a gene product to be expressed inappropriately or to assume a novel function, we do not know if the wild-type products of these genes are involved in vulval development. In *lin-24* and *lin-33* animals, which have a Vul phenotype, up to six of the 12 Pn.p cells die during the late L1 or early L2 stage (Table 2). (The surviving P(3–8).p cells sometimes express abnormal cell lineages during the L3 stage, moving dorsally within the ventral cord and generating up to eight nuclei with morphologies characteristic of neural cells.) These results suggest that the *lin-24* and *lin-33* mutations result in products with cytotoxic activities that disrupt the generation of the vulval precursor cells. In *lin-34* animals, which have a phenotype similar to that of *lin-15* or *lin-8; lin-9* animals, P(3,4,8).p express 1° or 2° lineages, but P(5–7).p express their normal lineages (Table 2), indicating that this mutation disrupts the determination of the 3° fate.

Our observations concerning two other genes—*lin-25* and *lin-31*—are inconsistent with their involvement at only a single step in the pathway of vulval development. In *lin-25* animals, which have a Vul phenotype, some of the Pn.p cells divide once during the L1 stage. In the L3 stage, most of the vulval cells (P(3–8).p or their L1-derived daughters) adopt 3° fates. The L1 defect of this mutant indicates that *lin-25* is active during the L1 stage in the formation of the vulval precursor cells. However, the TSP of *lin-25* extends to the middle of the L3 stage (Fig. 2), past the time at which the P(3–8).p cells divide, indicating that *lin-25* must also be involved in the expression of the vulval cell lineages. In *lin-31* animals, which have a Muv phenotype, some of the P(3–8).p cells divide during the late L2 stage to produce two to four descendants. In the L3 stage, the P(3–8).p cells or their L2-derived progeny can undergo three additional rounds of division, generating either vulval-like cells or compact neural-like cells. Thus, *lin-31* also may act at more than one of the steps of vulval development.

Conclusions

We have determined that the defects in vulval morphology resulting from mutations in any of 28 genes are caused by aberrations in the lineages of the cells that generate the vulva. Knowledge of the specific lineage defect in each strain has allowed us to assign 23 of these 28 genes to particular steps in the pathway of vulval development (Fig. 3). These assignments were confirmed by determining the epistatic interactions among mutations that affect different steps in the pathway and by analysing the temperature-sensitive periods of five of these genes.

Mutations in 19 of the genes that have been assigned to specific steps in this pathway cause certain cells to adopt fates characteristic of other cells. For example, mutations in three of the five genes necessary for the generation of the vulval precursor cells cause these cells to adopt fates characteristic of non-precursor cells: the *lin-26* mutation causes the normally hypodermal precursor cells to adopt neural fates; the mutation *n300* causes the precursor cells to adopt an alternative hypodermal fate; and the *lin-4* mutation apparently causes the precursor cells in the L3 stage to adopt a fate like that of their own progenitors during the L1 stage. Mutations in the 15 genes (see Fig. 3) that affect the determination of the fates of the precursor cells cause certain precursor cells to adopt fates characteristic of other precursor cells. Mutations in *lin-11*, one of the three genes that affect the expression of the 2° lineage, cause certain 2°-specific cells to adopt fates of other 2°-specific cells.

We consider these specific transformations in cell fates to be homoeotic, by analogy with insect mutants in which one body part is replaced by another part normally found elsewhere in the animal[17,18]. The existence of homoeotic vulval cell lineage mutants suggests that each step in the pathway of vulval development involves decisions that distinguish between alternative cell fates. Furthermore, there may be a regulatory hierarchy among the genes involved in these decisions. For example, *lin-12*, the activity of which is both necessary and sufficient for vulval precursor cells to become determined to express a 2° lineage, could act by regulating the activities of the genes involved in the expression of the 2° lineage, *lin-11*, *lin-17* and *lin-18*.

The nature of the transformations caused by these mutations suggests that we have identified genes involved in at least two possible mechanisms for specifying cell fates. First, mutations in *lin-11*, *lin-17*, *lin-18* and possibly *lin-26* disrupt or reverse the asymmetry of normally asymmetric cell divisions. If these divisions produce cells with autonomously determined fates, then these four genes may affect the generation of, segregation of, or response to determinants of cell fates that are normally asymmetrically distributed at certain cell divisions. Second, the 15 genes involved in the determination of the precursor cell fates act in the precursor cells in the reception of an intercellular signal and/or in the intracellular response to that signal. The discovery[19] that one of these genes, *lin-12*, encodes a protein homologous both to the precursor of mammalian epidermal growth factor and to the low-density lipoprotein receptor supports the hypothesis that *lin-12* acts as a component of an intercellular signalling system. Other genes involved in the intracellular response to the anchor cell signal might encode membrane or cytoplasmic proteins that function in 'second messenger' systems similar to those that have been characterized biochemically from other organisms[20–23] or nuclear proteins that function to control gene expression.

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Is 3C324 the first gravitationally lensed giant galaxy?

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The radiogalaxy 3C324 has recently raised considerable interest as one of the most distant (redshift $z = 1.206$) and luminous known galaxies. Here we report new observations of this object at Canada-France-Hawaii Telescope which confirm our previous predictions of the presence of an intervening gravitational lens. First, a multiple structure is revealed by our charge-coupled device (CCD) broad-band imagery. Second, following our previous identification in the 3C324 spectrum of an additional line system at $z = 0.84$, subsequent CCD observation through interferential filters were planned. In a wavelength range corresponding to an emission line at $z = 0.84$ only the central component is detected, while only outer components are seen in a range corresponding to a line redshifted by $z = 1.206$. These observations are interpreted as the first example of gravitational multiple imaging of a giant radiogalaxy by a foregound object. So galaxy 3C324 seems to be a gravitational mirage—though further spectroscopic observations will be needed to establish whether the two images have similar spectra.

On the basis of the confrontation of available data, with a theoretical analysis of the perturbations induced on the Hubble diagram by inhomogeneities in the actual Universe, two of us recently proposed that gravitational amplification of light affects far more extragalactic observations than currently believed[1-4]. Extrapolation of this analysis to the Spinrad and Djorgovski[5] very distant radiogalaxies led us to propose that their large observed overluminosity is at least partly due to strong gravitational lensing[6]. From case by case examination we have inferred the possibility of multiple imaging for some of them, including 3C324.

Based on our identification[6] in the high quality spectrum published by Spinrad and Djorgovski[7] of a line system (Ca II, H and K, and Hβ lines and MgH and MgB bands in absorption, [O II] in emission) due to a foreground galaxy at $z = 0.845 \pm 0.002$ superimposed on the narrow emission lines system at $z = 1.206$, new observations were planned.

Deep broad-band images were obtained by O.L. on 31 May and 1 June 1986 with the RCA2 CCD camera now available at Canada-France-Hawaii Telescope (CFHT)[8]. In spite of exposure times as long as 4,500 s in R (red wavelength) and 5,400 s in I (infrared wavelength), the full width at half maximum (FWHM) measured on stars is less than 0.7 arc s on both frames. In the R and I frames the multiple structure of 3C324 is evident[9]: two prominent components (a) and (b) separated by 1.1 arc s (12.9 h_{50}^{-1} kpc at $z = 1.206$, $q_0 = 0$) a fainter component (c) 0.8 arc s east of (a), and a probable faint component (d) 1.0 arc s north-west of (a) (Fig. 1*a*). Note also the two other galaxies (e) at 2.2 arc s from (b) and (f) at 3.0 arc s from (b). Calibrations on M92 field allowed us to derive the $R - I$ colour index for each component: $R - I_{(a)} = 0.65 \pm 0.20$, $R - I_{(b)} = 1.05 \pm 0.20$, $R - I_{(c)} = 1.10 \pm 0.30$, $R - I_{(d)} = 1.07 \pm 0.40$. A comparison with the spectroscopic data of Spinrad and Djorgovski in the [O II] λ3727 emission line at $z = 1.206$ (Fig. 1*d*) reveals that the (e) component, with a differential velocity around 1,000 km s^{-1}, is probably a galaxy at this redshift. Moreover, more than 100 galaxies are detected in only 1.15 × 1.86 arc min^2 confirming the hypothesis[7] of a cluster around 3C324.

On 6 August 1986 a 3,600 s exposure was obtained in a 90-Å width interferential filter centred at 5,139 Å, very close to the λ2335 [C II] emission line at $z = 1.206$ identified by Spinrad and Djorgorski. In this image (Fig. 1*b*) the measured seeing is 0.9 arc s: the components (b) and (c) only are detected while the brightest component in the broad-band image, (a), is obviously much fainter and probably non-emissive. The positions with respect to five stars in the field of both maxima in the R and 5,139 Å frames agree better than 0.13 arc s for (b) and 0.20 arc s for (c) (this larger error in the (c) position is due to the presence of the wings of (a) in the R frame).

On 7 and 8 August 1986 two 5,400 s exposures were obtained through an interferential filter with a 90 Å width centred at 6,840 Å, including [O II] λ3727 at $z = 0.845$. The seeings measured on field stars are respectively 0.8 and 0.85 arc s FWHM. Figure 1*c*, the digital sum of both frames after proper recentring, shows component (a) now prominent, while neither (b) nor (c) are detected down to the level expected from broad-band photometry. Both positions of (a) in the R and 6,840 Å frames agree better than 0.12 arc s.

From these data we conclude that the (a) component is a bright foreground galaxy at $z = 0.845$, possibly a spiral from its spectrum, axis ratio ~0.4 and $R - I = 0.65$, while components (b), (c) and (e) are objects at $z = 1.2$. Note also that (a) is on the line joining (b) to (c). Consider some possible interpretations of such a configuration. At first sight, the proximity of components (b), (c) and (e) could agree with the suggestion by Spinrad and Djorgovski[7] that some merging occurs in 3C324. But even if (b) and (c) were independent, the foreground galaxy (a) would act on (c) as a gravitational lens: from the condition of Subramanian and Cowlings[10], it would inevitably give multiple imaging provided that its mass is greater than $1.5 \times 10^{11} M_{\odot}$ inside 8 h_{50}^{-1} kpc, a condition probably fulfilled because (a) is 10 times brighter than our own galaxy. Therefore we conclude that the gravitational lens hypothesis is the most probable.

It is outside the scope of this report to present detailed modelling of the 3C324 lensing configuration, which would handle the theory of multiple imaging of an extended source by an extended lens. However, we intend to show that available data may already be accounted for by a very simple model where not only components (b) and (c) but also possibly component (d) are images of a source galaxy at $z = 1.206$ lensed by galaxy (a) at $z = 0.845$.

Consider the Bourassa and Kantowski[11] theory and its simple application to spheroidal transparent gravitational lenses characterized by a cutoff radius a_m. The image configuration depends on the relative value of two parameters: $\mu = (4G/c^2)(M/a_m^2)$ and $\sin^2\beta$. Here the usual distance term[11] D is equal to 0.074 c/H_0 and $\cos\beta$, the observed deflector axis ratio, may be tentatively estimated to be ~0.45 from the λ6,840 image. The facts that (b) and (c) components are dominant, lie on either sides and are aligned with the deflector, suggest that μ is large and that the lens behaves essentially as a point mass. Under this assumption[12,13], the image positions directly yield the critical radius $f = 10$ kpc and the source impact parameter $l = 4$ kpc, leading to a mass $M = 10^{12} M_{\odot}$, which is quite reasonable compared to the R absolute magnitude −21.5. Consider now the complete Bourassa and Kantowski[11] theory: asuming $a_m = 10$ kpc for the observed deflector extension implies $\mu \sim 1 \sim \sin^2\beta$. Depending on the line-of-sight position, one, three or five images may appear. There is very good agreement between the positions of the expected images 1 and 2 and of the components (b) and (c), accounting for the value previously derived for $l = 4$ kpc.

We may now compute the expected image amplifications in this model[14]. For component (b) and (c), we find respective amplifications $A_b = 1.80$ and $A_c = 1.0$. (Note that these values confirm the validity of our previous point mass approximation which would have given amplification factors of 1.82 and 0.82.)

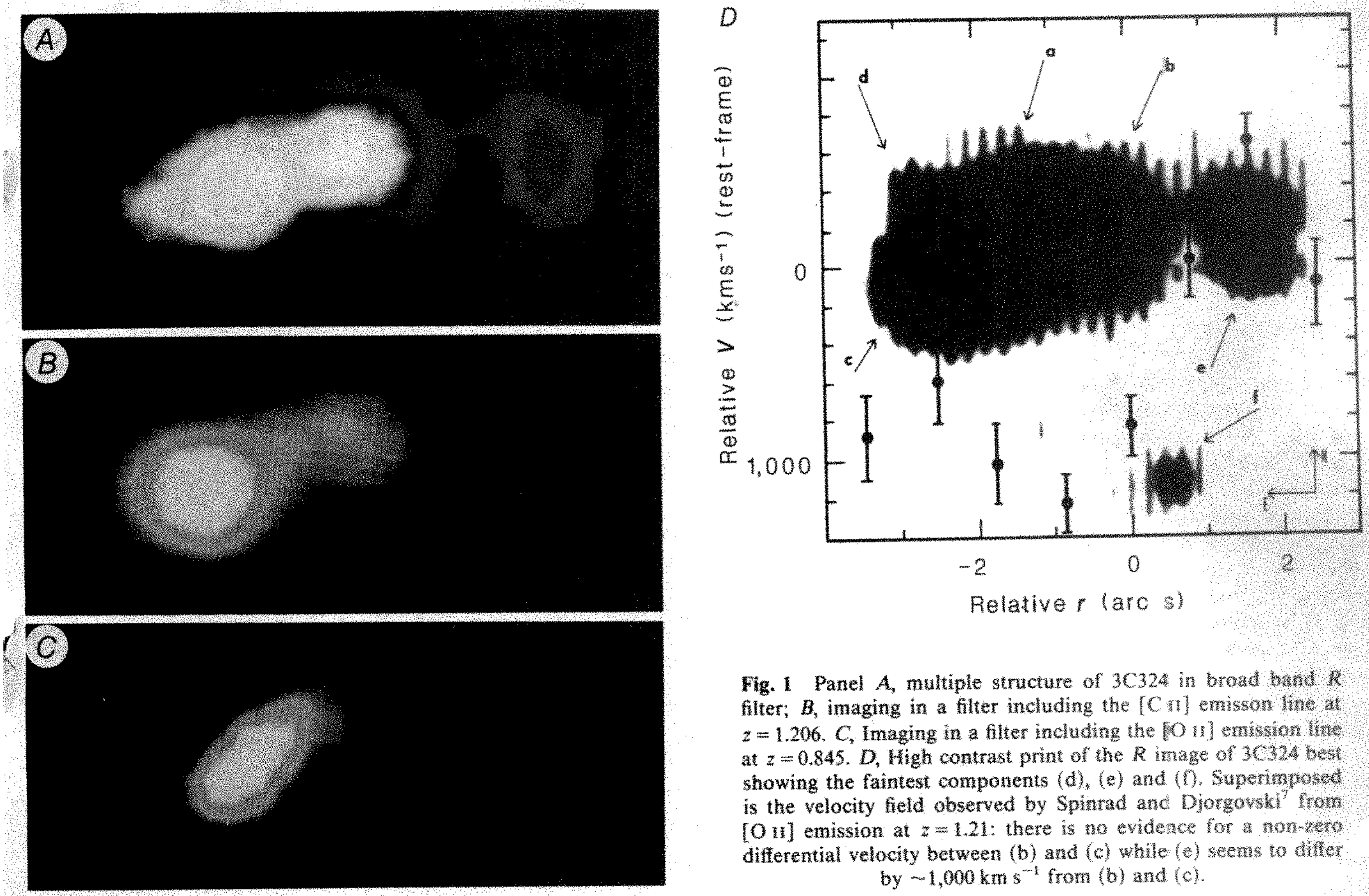

Fig. 1 Panel *A*, multiple structure of 3C324 in broad band *R* filter; *B*, imaging in a filter including the [C II] emisson line at $z = 1.206$. *C*, Imaging in a filter including the [O II] emission line at $z = 0.845$. *D*, High contrast print of the *R* image of 3C324 best showing the faintest components (d), (e) and (f). Superimposed is the velocity field observed by Spinrad and Djorgovski[7] from [O II] emission at $z = 1.21$: there is no evidence for a non-zero differential velocity between (b) and (c) while (e) seems to differ by ~1,000 km s^{-1} from (b) and (c).

The predicted amplifications agree well with *R* photometry which gives $R_b = 22.7$ and $R_c = 23.3$. As usual, the third expected image is so close to the deflector centre that overfocalization makes it unobservable.

Consider now component (d). It is quite remarkable that the observed axis ratio and position angle of deflector (a) have located it in the domain expected for images 4 and 5. Component (d) is, however, too faint for such an image position which predicts $d/c = 1.65$ instead of the observed value of 0.3. This discrepancy is easy to explain because, thanks to the source extension, a part of its outer region lies inside the diamond shaped figure of Bourassa and Kantowski[11] where five images are expected. Only 8% of the source light needs to fall within this limit to produce collapsed images 4 and 5 having the appearance of component (d). However, redshift information is clearly needed to confirm this configuration.

Other features of our data support our hypothesis, specifically the equality of the (b), (c) and (d) $R - I$ values (1.07), combined with their discrepancy from (a), $R - I$ (0.65), which agrees well with the gravitational lens interpretation of 3C324. Also, the model seems to exclude the presence of a rich galaxy cluster around the deflector at $z = 0.845$, which would drastically increase the luminosity ratio b/c. As indicated by the redshift of component (e) galaxy E of Spinrad and Djorgovski[5] and the high number of galaxies in the field, there is probably a cluster at $z = 1.2$.

In the λ5139 imagery, component (c) appears brighter than (b), contrary to what is observed in the *R* frame. We find that image (c) in λ5139 may be in fact a composite of (c), (d) and the continuum of (a) while in the λ6840 filter the (b) and (c) expected continua are ~1 mag fainter than (a). Only observations in stronger lines like [O II] λ3727 or Mg II λ2800 at $z = 1.206$ would definitively clarify this point.

Finally, in agreement with our previous suggestions[6], because of contamination by the foreground galaxy (a), and of total amplification by a factor of ~3, it is clear that 3C324 is subjected to a luminosity excess of ~2 magnitudes, and a colour excess of −0.2 magnitudes with respect to its real magnitude and $R - I$ colour index.

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The optical polarization of the Sun measured at a sensitivity of parts in ten million

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The integrated light of the Sun, an essentially spherical star with only slight asymmetries (small oblateness, weak overall magnetic field), would normally be found to be unpolarized, as observed in broad spectral bands with common instrumental sensitivities, 10^{-4}–10^{-5} fractional polarization[1–4]. Defining the Sun's intrinsic linear (LP) and circular (CP) polarizations down to much lower levels (10^{-7} or even 10^{-8}) would have consequences not only in solar physics but in other areas, setting, for example, a new standard for stellar polarimetry. We report measurements of the general polarization of the Sun, both CP and LP, over the whole disk and over large sectors, at an absolute sensitivity level of $\lesssim 3\times10^{-7}$, carried out during August–September 1986. Upper limits for the intrinsic whole-Sun LP from the best data (minimum Earth-atmospheric contamination) were 0.2×10^{-6} in the V (yellow) band and 0.8×10^{-6} in B (blue). Definite CP was discovered. (1) The north and south polar zones showed values of V (normalized or fractional CP) of -1 to -6×10^{-6} for the north, and 0 to $+2\times10^{-6}$ for the south zones. The spectral CP rises steeply toward the blue. (2) The whole disk had a net CP of -0.1 to -1.0×10^{-6} (from red to blue), negative as with the magnetically dominant north pole. The spectral dependence of the global broadband CP resembles that of sunspots[5,6] and of local non-spot regions with magnetic flux tubes[7].

An unusual arrangement was used for this high-sensitivity polarimetry of the whole Sun and large portions of its disk, shown in Fig. 1. The key element, a photoelastic modulator[3,8] (PEM) (manufactured by Hinds International, Portland, Oregon)—or sometimes a pair of PEMs—was mounted on the upper rim of the 81-cm telescope at Pine Mountain Observatory, Oregon. The telescope itself was used as a solar spar, with its main aperture blocked off by a shroud. The PEM faced the Sun directly, with no intervening optics which might cause spurious polarization, and below it were mounted an upper aperture plate; an optional lens; a stepping-motor rotated Polaroid analyser; a lower, image-plane aperture for use with the lens; a filter; and the detector, a blue-enhanced, 1 cm-diameter, flat-faced silicon photodiode, with associated preamplifier. The PEMs were fused-silica isometric types[3]. One or both of two units, of frequencies 32.4 and 35.1 kHz, were used. Detection and processing of the modulated polarized-light signals from the photodetector were done by standard electronics including a lock-in detector and computer[2]. Several spurious signals were cancelled by the rotating analyser (second stage chopper[2,8]) and by modulator rotation[8]. All data presented here are those averaged over four cardinal orientations of the analyser; and over four or eight orientations of the PEM or PEMs, using a rotator plate (Fig. 1).

For absolute polarimetry at levels $<10^{-6}$, certain instrumental effects not previously considered must be dealt with. In all polarimeters which employ birefringence modulators, including PEMs, Pockels cells and rotating wave plates, a fundamental aberration is caused by dichroic surface transmittance, which is inherent in Fresnel's laws of refraction at a dielectric interface. This imposes an oscillating linear polarization on the incident light, at the surfaces of the modulating device. In circular polarimetry, specifically, the CP is usually converted into a modulated LP by the device, and the LP is then converted into an intensity modulation by a stationary analyser-polarizer. The surface-dichroism effect produces a spurious, similar modulation. The false CP so induced can be as large as 10^{-4}. Because the linear polarizations produced by the real CP and the surface dichroism are 45° apart around the system axis, in principle the false signal can be eliminated by precise alignment of the analyser at 45° from the modulator eigen-axes. Owing to device imperfections this false signal cannot be cancelled with confidence by more than a factor of about 100, merely by device alignment. In our apparatus we have, instead, eliminated the false surface-dichroism signal by arranging for CP detection not at the basic modulator frequency, at which the false signal occurs, but at a different frequency. In one method, we employed two PEMs in tandem, operating at frequencies f_1 and f_2, with retardation amplitudes $\lambda/2$ and $\lambda/4$, respectively. This combination, together with the stationary analyser, produces a heterodyne CP signal in the detector of frequency $2f_1$-f_2; in our case this was 29.7 kHz. In a second method we used one PEM in a third-harmonic mode, with the retardation amplitude set at essentially $3\lambda/4$ and a third-harmonic CP signal (frequency $3f_1$) was generated. Some of the CP data reported here, namely the data of 7–8 August in Fig. 2 and 27 July in Fig. 3, were obtained with the heterodyne ($2f_1$-f_2) method; all the other data in Figs 2–3 were obtained with the $3f$ method. No systematic difference is seen between the results with these different arrangements.

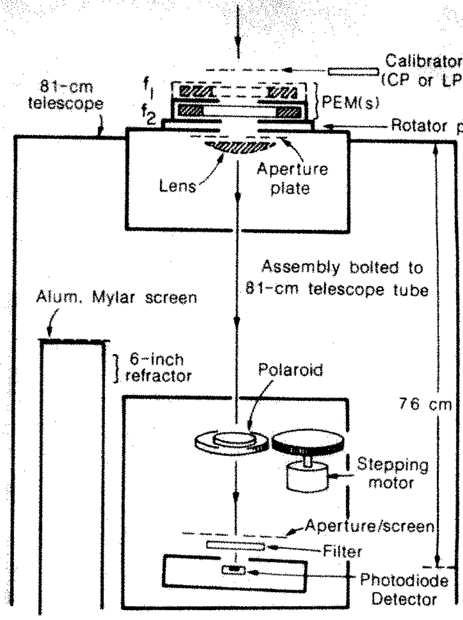

Fig. 1 Solar polarimeter assembly attached to the 81-cm telescope at Pine Mountain Observatory, Oregon. Telescope was used only as a solar spar, with entrance covered. Guiding was done with the 6-inch guide telescope, and by directly viewing the image on the detector. Apparatus was accessed from a scaffold erected by the telescope. All measurements involved successive measures with the photoelastic modulator (PEM) assembly rotated to four or eight cardinal positions around the system axis, and with the polaroid analyser rotated to four positions, to cancel out various instrumental signals. Lens was used for sector measurements (Fig. 2); no lens was used in the whole-disk observations.

Linear polarimetry was performed by the second-harmonic method[9], which requires rotating a single PEM to two positions, 45° apart, to obtain the two linear Stokes parameters Q and U. In fact we used eight cardinal orientations, with suitable averaging. In the LP case, second-harmonic detection immediately eliminates false signals due to the surface-dichroism effect discussed above. In our apparatus the only instrumental LP was judged to be caused by slight misalignment of the system from

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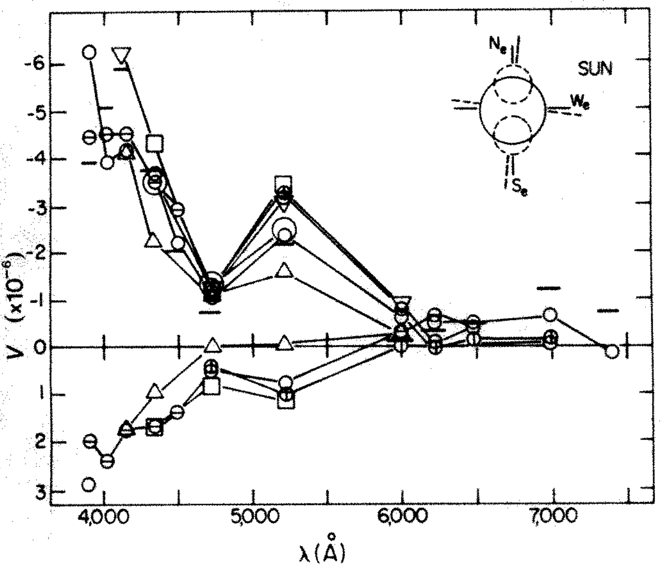

Fig. 2 Filter-band measures of the Sun's circular polarization, for the northpole, *a*, and the south pole, *b*, using a 15 arc min-diameter aperture at the focal plane over the photodetector. Approximate positions on the Sun's disk are sketched at upper right; positions were over the ecliptic poles, N_e and S_e, which were rotated slightly from terrestrial north and south (which are indicated by broken lines) during the observing period. Horizontal line segments (without symols), approximate CP values of a typical sunspot, extracted from data in ref. 5, for interference filter bands equivalent to those used in this work; these values are scaled arbitrarily on the vertical scale for visual comparison with the north pole data. The ordinate scale here is in units of 10^{-6} fractional polarization. Filter widths used were from 60-200 Å. For brevity, in Figs 2 and 3 and in the text, V denotes the normalized quantity V/I, in terms of Stokes parameters. ○, 7 August; ⌽, 10 August; ◯, 15 August; ∇, 2 September; ⊖, 8 August; □, 11 August; △, 22-23 August (1986).

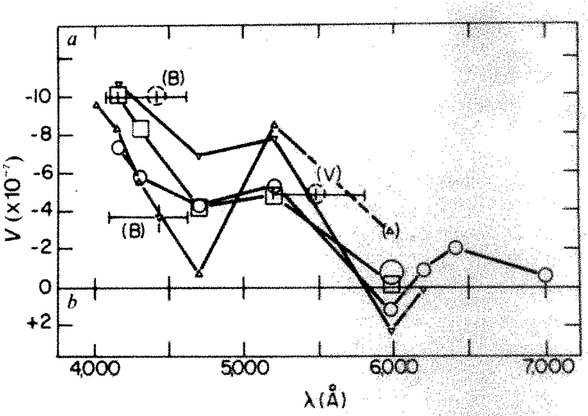

Fig. 3 The circular polarization of the Sun's whole disk. No lens was used, and an angular aperture of diameter 2° was defined only by the upper aperture plate in Fig. 1. Although we do not show individual measurement errors, the formal errors merely from photon statistics are mostly small compared to the scatter between points from different dates, caused by instrumental polarization changes and possibly changes in the Sun's polarization. Note that the vertical (fractional CP) scale here is in untis fo 10^{-7}, rather than 10^{-6} as in Fig. 2. The measurements were taken on: ○, 27 July; △, 16 August; ○, 19 August; □, 21 August; ∇, 24 August; ◯, 2 September (1986).

collinearity with the light axis. The spurious LP is of the order of 0.1 θ^2, where θ is the misalignment angle of the PEM surface[3]. In our case we estimated that $\theta \leq 0.1° = 0.0017$ rad, implying that $p_{\text{inst}} \leq 3 \times 10^{-7}$. As the Sun is an extended object, this estimate applies most specifically to whole-disk observations with the Sun centred in the system aperture.

Many tests and modifications of the apparatus were made during early phases of this project, for example, the elimination of inter-reflections, which we accomplished by slightly tilting the analyser and filter-detector assemblies (Fig. 1). At a suggestion by J. Stenflo (personal communication) we experimented with an auxiliary 'scrambler' PEM[10], placed above the main PEM, which in theory can eliminate the real signal and reveal spurious ones. We found that the scrambler itself introduced new false signals. Our general conclusion is that no optical components of any kind should intervene between the observed source (the Sun) and the principal polarimeter element. No light source known to be unpolarized at the 10^{-7} level was available to test this system. Nevertheless, based on intimate knowledge of the apparatus and on consistencies in the final data, we believe that the instrumental levels in most of the CP and LP data here reported (Figs 2-4) do not exceed $\sim 3 \times 10^{-7}$. Even greater sensitivity is possible in future work.

In Figs 2 and 3 we show north and south and global measures of the Sun's broadband CP, given in terms of the normalized polarization (circularly polarized flux divided by the total flux). The north and south polar measurements were made with an aperture diameter of 15 arc min, with approximate positions as shown in Fig. 2. In the whole-disk measures, Fig. 3, the effective aperture was 2°. Interference filters of various widths, 60-200 Å, were used. The north-south measurements were emphasized partly because the north-south dipolar magnetic field is strong at this time in the solar cycle, close to sunspot minimum. A few east-west measurements were taken, using the aperture as in Fig. 2 rotated to the east and west limbs, all in blue filter bands (see Table 1). No sunspots were evident in the north-south measurements. The few spots seen during the series were near the equator, as is typical near sunspot minimum. One or two of the east-west measurements may have been influenced by spots. The CP quantity V in Figs 2 and 3, and in Table 1, is the normalized circular Stokes parameter (divided by the intensity I). For the signs of V we use the helicity convention: positive V means that, in a stationary plane in front of the observer, the **E** vector is seen to rotate counter-clockwise.

In Fig. 1 note: (1) a marked wavelength dependence, including a general rise into the blue ultraviolet and a dip around 4,700 Å. Such structure, especially the 4,700-Å drop, cannot be of instrumental origin and is proof that we are detecting real polarization; (2) a dominance of the Sun's CP by the north sector. Regarding the spectral dependence we include in Fig. 2 horizontal line segments showing equivalent sunspot CP values, in filter bands comparable to those used in the present sector measures, extracted from Fig. 2 of Kemp and Henson[5]. An arbitrary sign and an arbitrary scale are used for the sunspot CP values, which are ~100 times larger than the north-south sector values. The similarity in spectrum is striking. In the whole-disk data of Fig. 3, a basically similar wavelength dependence is seen, although the values are 5-10 times smaller than in the north aperture of Fig. 2, and the data show considerable scatter. Clearly the negative sign of the whole-disk values reflects the dominance of the north

Table 1 East-west circular polarization measures of the sun

Date 1986	Filter centre (Å)	V (east) ($\times 10^{-6}$)	V (west) ($\times 10^{-6}$)	$(V_E - V_W)/2$ ($\times 10^{-6}$)
2 August	4,300 (B)	−0.17	+0.48	−0.32
3 August	4,300 (B)	−2.76	+1.84	−2.30
5 August	4,300 (B)	−2.46	+1.41	−1.94
11 August	4,306	−2.14	+0.05	−1.09
23 August	4,150	−0.26	−3.01	+1.40
24 August	4,150	+0.89	−2.11	+1.50
2 September	4,150	+1.46	+0.34	+0.56

15 arc min-diameter aperture, positions analogous to north-south positions in Fig. 2.

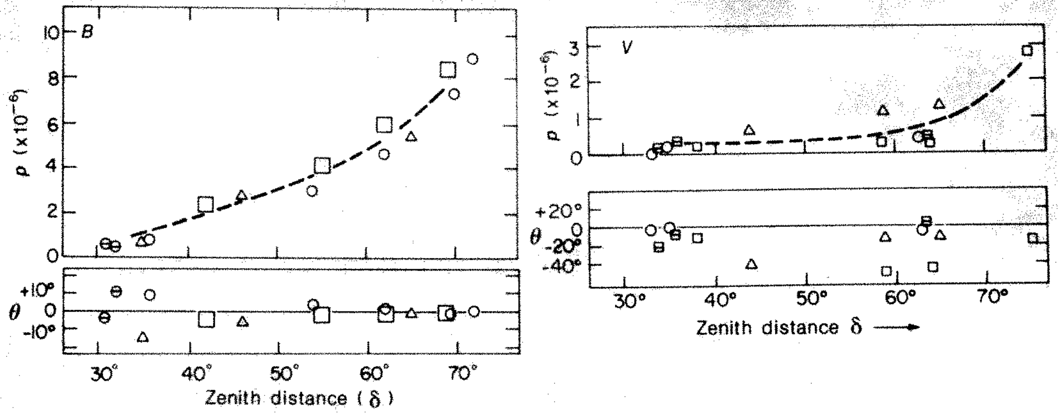

Fig. 4 Measured values of the Sun's whole-disk linear polarization, obtained as in the CP measurements of Fig. 3 without imaging (no lens), using a 2° aperture. Standard astronomical *B* and *V* filters (mean wavelengths 4,400 Å and 5,530 Å respectively) were used. The vertical scales for *p* (polarization amplitude) are in units of 10^{-6} fractional LP, We show $p(\delta)$ and $\theta'(\delta)$, where δ is the zenith distance (Sun-zenith angle) and $\theta' = \theta - \theta_{zen}$ is the difference between the position angle of the Sun's observed polarization in the equatorial system, and the Sun-zenith direction on the sky. At large zenith angles the observed LP, at least in the blue band, is almost entirely controlled by atmospheric scattering. In the *V* band, owing to the very small *p* values the position angles were very poorly defined. For the *B* filter diagram the measurements were taken on: ⊖, 12 August (a.m.); ○, 12 August (p.m.); △, 25 August (a.m.); □, 26 August (a.m.); (1986). For the *V* filter diagram the measurements were taken on: ○, 10 August (a.m.); ⊟, 12 August (a.m.); □, 12 August (p.m.); △, 26 August (a.m.) (1986).

polar CP. Note that these data span roughly a rotation period of the Sun, so that azimuthal structure can be largely averaged out. In these CP measurements there was no indication of Earth-atmospheric influences, such as zenith-distance variation.

Our linear polarization results, in contrast to the CP measures, were controlled mainly by terrestrial atmospheric effects. Only whole-disk measures were taken, in 1°- and 2°-diameter apertures, in the *B* and *V* bands. The observed LP correlated in magnitude and position angle with the Sun's position on the sky, at least in the *B* band wherein the LP was strongest and better defined. In Fig. 4 the LP is shown in terms of the magnitude *p* (in units of 10^{-6} fractional LP); and a position angle $\theta' = \theta - \theta_{zen}$, where θ is the actual position angle (E vector direction) in the standard equatorial system and θ_{zen} is the position angle of the Sun-zenith direction. Thus $\theta' = 0$ means that the E vector is along the Sun-zenith great circle. Such LP is termed 'negative' in the terminology of terrestrial sky polarization[11]. This negative LP, increasing with zenith distance, is attributed to double scattering by aerosols and molecules in the Earth's atmosphere[11]. Using a 1° aperture we also found that the adjacent sky around the Sun, in the so-called aureole[12], had comparable LP flux as in the Sun measures, with the same position angle. We conclude from Fig. 4 that the Sun's intrinsic whole-disk LP does not exceed the levels at the smallest zenith angles, that is, about 0.8×10^{-6} in *B* and 0.2×10^{-6} in *V*. Better LP measures should be taken at the zenith (at a low-latitude site) or in space.

The polar circular polarization, with opposing signs north and south, is probably a manifestation of the Sun's dipolar magnetic field, which is approximately at maximum strength now (sunspot minimum). A referee has noted that the northern and southern hemispheres had different magnetic structures during this period, the northern hemisphere having a smooth appearance whereas the southern hemisphere was magnetically complex. In any case, however, the northern zone during 1986 was magnetically negative, as shown for example by the Stanford magnetograph measures (ref. 13 and 1986 data supplied by P. Scherrer and J. Hoeksema), whereas the south pole was positive. Thus the sign relationship here is that our negative broadband *V* in the northern zone corresponds to a predominant negative (inward) field, and vice versa in the south. This sign correlation will be one test of theories of the global CP.

Regarding the origin of the global CP, as noted above the coarse spectrum (Figs 2 and 3) is rather similar to the broadband CP spectrum of sunspots[5] and also to the CP spectrum of mildly magnetic regions away from spots, containing flux tubes (Fig. 5 of ref. 7). Thus a common mechanism for the CP is indicated. The coarse $V(\lambda)$ spectra reflect the photospheric line blanketing, which generally rises toward the blue, apart from local (essentially statistical) irregularities. The source of the broadband CP obviously lies in the spectral lines. Kemp and Henson[5] showed that in sunspots the continuum contribution, from grey magneto-opacity, is very weak in visible bands and is significant only in the infrared. In the case of broadband CP observed in flux-tube regions away from spots, it has been shown[7] that this is an aspect of asymmetries between the blue and red peaks of the $V(\lambda)$ curves of individual lines[14,15], which have opposite polarities but systematically different total strengths. The most quoted candidate source for this asymmetry, and for the resultant broadband CP, is mass motion in the flux tubes. Correlated vertical field and velocity gradients, causing differing Zeeman and Doppler shifts at different depths, combine with line saturation to produce the asymmetry[16]. At present there are several problems with quantitative models of this as discussed recently by Stenflo *et al.*[17].

The Sun's surface is known to be threaded by a network of magnetic flux tubes, of sub-arc s diameters, with generally alternating polarities (see, for example ref. 18). Large-sector and global broadband CP must arise, in a manner of speaking, from a slight excess of tubes of one polarity, which account for an average vector field component in the observer's direction. Regarding the magnitudes of our observed global CP, the vector line-of-sight field in our polar-sector measures (Fig. 2) can be gauged from magnetograph data[13] as perhaps being some 2 gauss in either polar region. From sunspot *V* values near 4,100 Å of $\sim 6 \times 10^{-4}$ with 2 kilogauss fields[5], we might anticipate $V \sim 1 \times 10^{-6}$ in our north or south apertures, which is a few times smaller than we observe around this wavelength (Fig. 2). On the other hand a rough scaling based on typical Zeeman line asymmetries observed in non-spot regions (J. W. Harvey, personal communication), assuming reasonable area filling factors for the flux tubes, seems consistent with a *V* of a few parts in 10^6 as we observe.

The signs of our measured *V* values, with given polarity of the vector line-of-sight fields, should be compared with the signs expected from Zeeman line-wing asymmetries in magnetic non-spot regions[7,14]. In all areas well away from the limb, the latter predict a negative broadband *V* in a region with positive (outward) field, and conversely. (We adhere to the helicity sign convention defined above.) In our north-south measures (Fig. 2), we see just the opposite. However, it has been recently found that near the solar limb, for $\mu = \cos\theta \leq 0.4$ where θ is the angle between the surface normal and the observer's direction, the line-wing area asymmetries change sign[17]. Evidently, in our north-south apertures the reversed 'limb' sign predominates. The polarized-light radiative transfer in flux tubes on inclined surfaces is clearly complex, and more is involved than merely the line-of-sight field and its polarity[17]. In spite of this complexity, there can be no doubt that the broadband circular polarization mirrors, semi-quantitatively, the magnetic field structure, as has been suggested[7].

The whole-disk CP of Fig. 3 might reflect only a transient condition of the Sun in which the northern hemisphere was dominated by a relatively ordered field. Or, it might be caused by the tilt of the Sun's spin axis combined with the north-south dipolar field. On about 8 September 1986 the Sun's north pole was inclined at its maximum angle (7.2°) towards the Earth[19]. Throughout August it was inclined at least 5.7° towards the Earth, which could explain the dominance of the north polar CP. Whole-disk magnetographs during August 1986 (J. T. Hoeksema and P. H. Scherrer, personal communication) confirmed an average negative magnetic polarity for the period. It is not clear how the rather small tilt angle of the Sun's axis could produce such a sizeable whole-disk CP and such a large difference in the north and south polar measures. Observations over years will be needed to investigate this.

A net circular polarization in the Sun's light, averaged over the whole visible spectrum, has implications in areas other than solar physics. In the solar-terrestrial connection, we wonder whether a quite small circular polarization might have influenced the chirality (average handedness) of the Earth's biomolecules. In the light received by the Earth, the Sun's overall CP would tend to average to zero over long periods, owing to both the Earth's orbital motion around the Sun's tilted spin axis and the reversal, every 11 years, of the Sun's dipolar field. On timescales of 10^9 years, there would seem to be no net effect. However, if biogenesis began not slowly but in a burst of photochemistry, over a period of months perhaps, then a net CP in sunlight might have promoted chirality. It is not clear whether a CP as small as 10^{-6} could have been effective in this connection. The structure, strength and periodicity of the Sun's global magnetic field might have been quite different 3 Gyr ago.

Magnetic fields in solar and late-type stars, associated with star spots and related phenomena, may now be profitably looked for by the broadband CP method, presuming that the same process underlying the Sun's global magnetic CP operates in similar stars. Searches for magnetic fields in late-type stars have largely used indirect, non-polarimetric methods such as the sensing of Zeeman broadening of spectral lines[20,21]. Scaling from our solar results, we note that a 10-gauss line-of-site vector field in such stars would produce a broadband CP of possibly 10^{-5}, which we have shown to be readily detectable. In the case of one of the brightest of these stars, the star Lambda Andromedae, for which there is strong evidence for Zeeman line broadening[21], we will soon submit (Kemp *et al.*, in preparation) a report on the direct detection of broadband circular polarization.

This unusual project and the interpretation benefited greatly from the encouragement and advice of colleagues including Jack Harvey, A. Skumanich, Jan Stenflo, Phil Scherrer and S. Solanki. Ted Kinsman aided in some of the measurements. We received support from NSF grant ATM 841176 and the National Geographic Society grant 3003-84.

Note added in proof: We call attention to an important new paper on the theory of broadband polarization in the Sun and sunspots by A. Skumanich and B. W. Lites, *Astrophys. J.* (submitted).

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Evidence for two intervals of enhanced ^{10}Be deposition in Antarctic ice during the last glacial period

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We have previously reported concentration profiles of cosmic ray produced (cosmogenic) ^{10}Be in deep ice cores from Dome C and Vostok, Antarctica[1,2]. In both these cores we found a concentration of ^{10}Be approximately two times larger in ice from the late glacial period than in the Holocene ice. We interpreted this as probably resulting from a lower precipitation rate on the Antarctic plateau during glacial periods, compared to interglacial periods. In the Vostok profile there was one sample, corresponding to ~60,000 yr BP, which gave an unusually large ^{10}Be concentration, not correlated with any obvious climatic event. We suggested that this sample might be reflecting increased ^{10}Be production, as for example during a period of reduced solar modulation[2]. We have now measured a much more detailed concentration profile for ^{10}Be in the Vostok core. The results confirm a ^{10}Be 'peak', lasting ~1,000–2,000 years, at ~60,000 yr BP, and show another similar peak at ~35,000 yr BP. We have also observed the latter peak in the Dome C core. We discuss possible sources for these peaks, and their potential as stratigraphic markers.

Our previous Vostok profile was based on samples from 22 different depth levels[2]. We now have measurements for 92 levels, including almost all those used for the oxygen isotope profile of ref. 3. In addition we have subdivided the samples from a number of the levels (including the two 'peaks') into two or more fractions, to obtain some information on short-term fluctuations in the ^{10}Be concentration. The accelerator mass spectrometry measurements of ^{10}Be were carried out as described in ref. 2, with the exception that in this work only 0.25 mg of ^{9}Be carrier was used. For virtually all the new samples, 1,000 or more ^{10}Be nuclei were recorded, making the one standard deviation statistical contribution to the measurement uncertainty <3%. Better stability in our operating conditions has also allowed us to reduce from 7 to 5% the instrumental contribution to our estimated uncertainties for most of the new measurements.

The multiple measurements for those samples subdivided, together with the length and estimated deposition time of the individual subsamples, are summarized in Table 1. It can be seen that in most cases the concentrations in the subsamples agree to within 10% of that for the mean concentration at that level. There are, however, exceptions in which the variability is considerably larger, perhaps due to short-term precipitation

Table 1 ^{10}Be concentration in levels subsampled

Sample designation	Nominal depth* (m)	Sample length (cm)	Estimated deposition interval† (yr)	^{10}Be conc. ($\times 10^3$ atoms g^{-1})	Mean ^{10}Be for level ($\times 10^3$ atoms g^{-1})
1c	25	18	7	97±5	89±5
1d	25	15	6	79±4	
10f (1)	250	11	5	87±7	86±6
(2)	250	11	5	86±5	
12c (1)	300	12	6	100±6	113±6
(2)	300	12	6	125±7	
18b (1)	450	9	8	211±11	218±12
(2)	450	9	8	227±12	
19a (1)	475	13	11	162±8	180±9
(2)	475	13	11	167±9	
(3)	475	13	11	204±10	
23d (1)	575	7	6	162±8	193±10
(2)	575	7	6	176±9	
(3)	575	7	6	243±12	
24a (1)	600	12	10	346±18	316±16
(2)	600	12	10	283±15	
26d (1)	650	8	7	221±12	208±11
(2)	650	8	7	196±11	
29b	725	14	11	156±8	152±8
29d	725	11	8	146±8	
37a	925	25	21	266±19	269±20
37b (1)	925	7	6	212±17	
(2)	925	6	5	231±21	
(3)	925	7	6	302±21	
(4)	925	6	5	342±27	
38e (1)	950	7	6	136±10	139±10
(2)	950	7	6	141±11	
(3)	950	9	8	141±11	
40b (1)	1,000	8	7	180±10	171±9
(2)	1,000	8	7	162±9	
42a (1)	1,050	11	10	214±11	196±10
(2)	1,050	11	10	175±9	
43a (1)	1,075	10	9	113±6	118±7
(2)	1,075	10	9	125±9	
44b (1)	1,100	9	7	114±6	134±7
(2)	1,100	9	7	154±8	
48b (1)	1,200	10	8	114±6	114±6
(2)	1,200	10	8	114±6	
54a (1)	1,350	15	12	167±9	162±9
(2)	1,350	15	12	157±9	
62d (1)	1,550	21	20	132±7	140±8
(2)	1,550	19	18	152±8	
63c (1)	1,575	10	10	142±8	119±7
(2)	1,575	10	10	93±5	
65a (1)	1,625	10	9	116±6	125±7
(2)	1,625	10	9	134±7	
67f	1,675	10	7	68±4	72±4
67j	1,675	10	7	77±4	
69a	1,725	15	11	68±4	72±4
69d	1,725	10	7	77±4	
70b (1)	1,750	16	12	64±4	70±4
(2)	1,750	16	12	77±4	
70c	1,750	20	15	72±6	
71f (1)	1,775	11	8	74±4	76±4
(2)	1,775	11	8	78±4	
81b (1)	2,025	12	21	98±5	104±5
(2)	2,025	14	25	110±6	
92c (1)	2,050	20	35	123±6	127±7
(2)	2,050	20	35	131±8	

* Correct to ±2 m.

† Estimated from chronology in ref. 3.

variations, or short-term solar modulation variations. For levels that were not subdivided, we have always tried to take samples corresponding to at least 10 yr of precipitation, in order to minimize the above effects. We thus believe that in general our measurements are reasonably representative of the ^{10}Be concentration at the levels sampled, although there may be occasional exceptions.

Our results, including those previously published[2], are shown in Fig. 1. For levels for which we have multiple measurements, we have plotted the weighted average value. The error bars indicate only estimated measurement uncertainties. As before[2] we have plotted the ^{10}Be on an inverted scale to facilitate comparison with the oxygen isotope curve[3]. The ^{10}Be profile in Fig. 1 in general confirms the trends noted in ref. 2: in particular

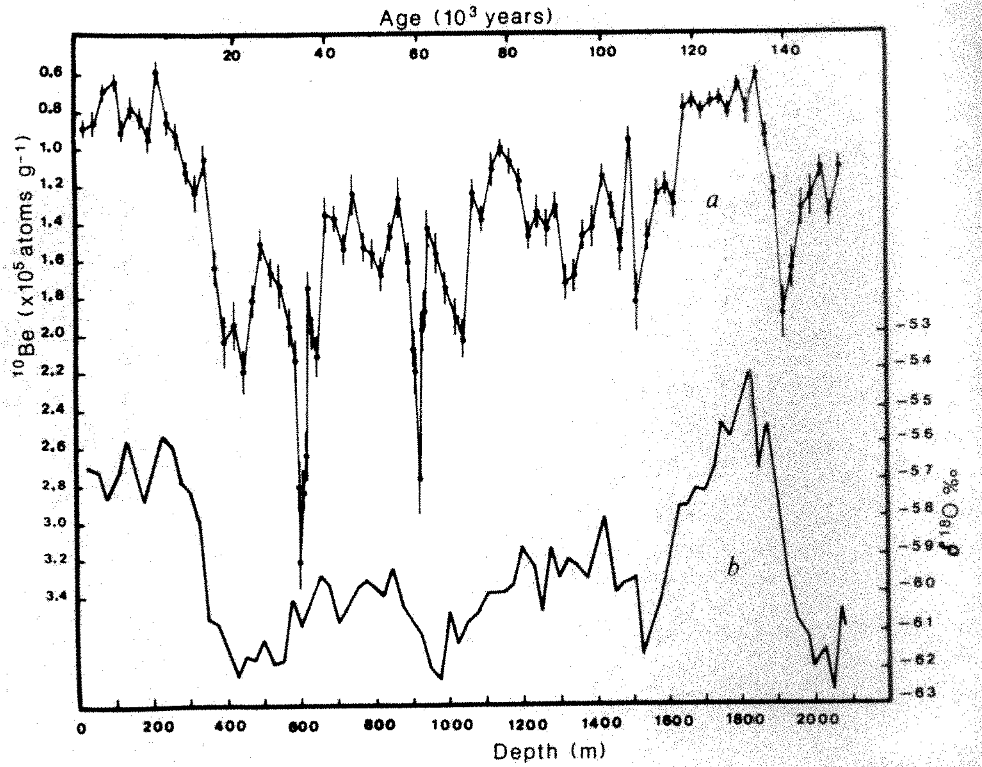

Fig. 1 *a*, The ^{10}Be concentration and *b*, δ^{18}O as a function of depth in Vostok ice core. Note inverted scale for ^{10}Be to facilitate comparison between the two curves. Small corrections (<7%) for ^{10}Be decay have been made using the timescale (top abscissa) adopted by Lorius *et al.*[3].

note the good correlation with the oxygen isotope curve, and the factor of ~2 larger ^{10}Be concentration during the glacial period compared to the Holocene and previous interglacial. In addition the greater detail of the present profile allows us to observe more subtle effects. For example, the ^{10}Be concentration appears to increase quite steadily from the previous interglacial until the glacial maximum at ~20,000 yr BP, followed by a rather rapid decrease to Holocene levels. Similar 'saw-tooth' structure is often observed in other climatological curves for this period[4], although it is not particularly obvious in the published Vostok oxygen isotope record[3]. Also, there appear to be some differences in detail between the ^{10}Be and δ^{18}O curves for the period just before the previous interglacial, with the maximum in the ^{10}Be record being apparently much narrower than that of the δ^{18}O. We will not comment further on these points here.

The most dramatic difference between the ^{10}Be and δ^{18}O curves are the two fairly well defined peaks in the ^{10}Be concentration, at ~925 m and ~600 m. According to the timescale adopted by Lorius *et al.*[3], these levels correspond to times of ~60,000 and ~35,000 yr BP, respectively. Each of the peaks is estimated to last ~1,000–2,000 years, depending on the assumed precipitation rate and how one chooses to define the peak shapes.

Once we discovered the ^{10}Be peak in the Vostok core at 600 m, we decided to measure ^{10}Be in samples from the same time period in the Dome C core, as estimated from a comparison of continuous δ^{18}O profiles in the two cores (the second peak at 925 m in the Vostok core is beyond the range covered by the Dome C core). A comparison of the Vostok and Dome C profiles is shown in Fig. 2. It is clear that both the relative amplitude and widths of the ^{10}Be peaks are very similar in the two cores. We believe this good correlation excludes experimental artefacts, or local geographical effects, as the cause of the observed ^{10}Be concentration increases. However, the fact that both these sites are on the Antarctic plateau, and subject to similar meteorological conditions, means that we cannot exclude regional climatic effects as a possible common cause of the peaks, as discussed below.

The first question we must address is whether the observed ^{10}Be concentration peaks are actually due to increased ^{10}Be deposition, or whether they are simply more dramatic examples of the precipitation variation effect mentioned earlier as being the probable cause of the glacial–interglacial concentration differences. There are two arguments against this explanation. The first is that, according to our working hypothesis, which appears to be consistent with all the data collected so far[1–3] the precipitation rate at these two sites seems to be controlled mainly by the water vapour pressure, and thus temperature, above the tropospheric inversion layer. The δ^{18}O data show no evidence for any major change in this temperature for the periods concerned. Second, and perhaps more convincing, any decrease in precipitation rate would be expected to show up also in increased concentrations of other aerosol components, such as dust and trace elements, for these periods. There is, however, no evidence for particular enhancements of these other components at these depths[5,6]. Although both aluminum[5] and microparticles (J. R. Petit, personal communication) show a concentration maximum at ~900–1,000 m in the Vostok core, the width of the peak is considerably larger than that of the ^{10}Be. We thus conclude that the ^{10}Be peaks probably do reflect real increases in ^{10}Be deposition.

We have previously discussed two other potential causes for increased ^{10}Be concentration in polar ice[1]. One of these is increased ^{10}Be deposition due to changes in atmospheric circulation. We estimate that only a rather small fraction of stratospherically produced ^{10}Be is presently deposited on the Antarctic plateau[7], it is quite easy to imagine changes in this fraction giving rise to peaks of the type observed. One might again cite the absence of any correlation with other trace components in the ice as an argument against such an explanation. However, the source of these other components is quite different from ^{10}Be, so such an argument cannot be conclusive. Thus, although there is no evidence for changing atmospheric circulation as the source of the ^{10}Be peaks, ultimately the only way of excluding this explanation will be to find similar peaks in other reservoirs.

The third potential source of the ^{10}Be peaks, and the one we favour for the moment, is that they are due to increased production of ^{10}Be in the atmosphere. There are three possible causes for such production changes[8]: (1) variations in primary cosmic ray flux, (2) changes in solar modulation, (3) changes in geomagnetic field intensity.

Although there is still no general agreement on the source of galactic cosmic rays, it seems unlikely to us that variations of

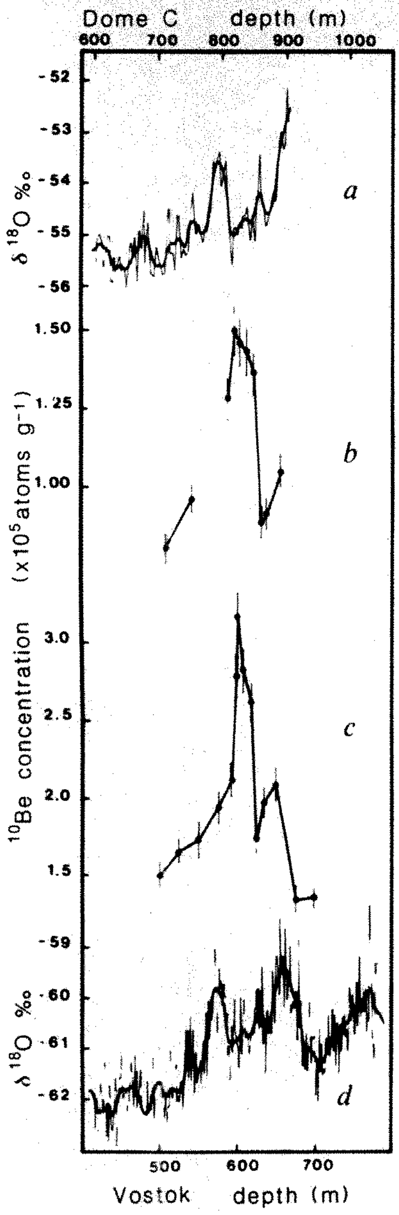

Fig. 2 *a*, δ^{18}O and *b*, ^{10}Be concentration in Dome C core; *c*, ^{10}Be concentration and *d*, δ^{18}O in Vostok core. For δ^{18}O the fine lines represent raw data, dark curves are fits with second order spline functions. The depths in the two cores have been correlated by the δ^{18}O curves, in particular the peaks at ~575 m and 650 m on the Vostok depth scale.

the magnitude necessary to produce the observed ^{10}Be peaks could take place on the timescales involved.

The reason we originally suggested[2] the possibility of changes in solar activity (through solar modulation) as the possible source of the observed ^{10}Be peak at 925 m (before we had any indication of its duration), is because changes in modulation are well documented at the ~11 yr timescale (solar sunspot cycle), and are also believed to be responsible for observed ^{14}C variations observed on timescales of ~100-200 years during the Holocene[9,10]. Variations in ^{10}Be on similar timescales have also been attributed to this cause[1,11,12]. If the ^{10}Be peaks presented here are due to changes in solar modulation, then they would represent evidence of reduced solar activity for much longer periods than previously documented. This could potentially have important implications for models of solar variability, and solar-terrestrial relationships.

The third possible source of ^{10}Be production variations are changes in the geomagnetic field intensity. It is widely believed that such intensity changes were responsible for the long-term changes in the ^{14}C/^{12}C ratio in the atmosphere during the past ~8,000 years[13]. However, Beer *et al.*[12] have reported the absence of any corresponding variation in ^{10}Be in Greenland ice for the same time period. In order to increase global ^{10}Be production by a factor of ~2, it would be necessary for the geomagnetic field intensity to fall to <20% of its present value[14]. Reductions of this magnitude are known to occur during geomagnetic reversals. We have in fact reported increased ^{10}Be concentrations in a marine sediment core at the time of the most recent of these reversals 700,000 years ago[15]. In addition to well recognized global reversals, there are numerous reports in the literature of geomagnetic 'excursions', periods during which the apparent dipole direction and/or intensity at a given location fluctuate strongly from their 'normal' values for periods of a few hundred to a few thousand years[16]. It should be emphasized, however, that there is no general agreement that any of these recorded excursions actually represent global changes in the geomagnetic field[16,17]. In fact we have pointed out that ^{10}Be could be an important probe for testing the global nature of excursions[15] (no significant changes in ^{10}Be production would be expected for only local or regional geomagnetic variations). Interestingly, several of the reported excursions (Laschamp[18], Mono Lake[19,20], Lake Mungo[21], Shibutami[22]) have estimated ages in the range of 25,000-45,000 yr BP. It is thus natural to speculate whether the ^{10}Be peak observed here at ~35,000 yr BP could be caused by low fields associated with one or more of these excursions. (There is as yet no convincing evidence that any of these reported excursions are related[16,17].) There is at least one possible argument against such an explanation, and this is that the influence of the geomagnetic field on ^{10}Be production is strongest in the equatorial region, and essentially zero near the poles. Indeed, we have noted[7] that this might be the reason that Beer *et al.*[12] did not observe, at Camp Century Greenland, the variation of ^{10}Be expected for a dipole change sufficient to give the observed long term variation of ^{14}C in the atmosphere during the Holocene. The ^{10}Be peaks observed here are larger than the calculated production increase in the polar regions, even for a dipole intensity of zero[14]. Thus, if geomagnetic variations are the source of the ^{10}Be peaks, it would imply that, at least during those periods, there was significant transport of lower latitude aerosols to the Antarctic plateau.

It is clear that considerably more work will be necessary to understand fully the implications of the periods of high ^{10}Be deposition presented here. Perhaps most urgent will be the research to try to find evidence of these periods in other geological reservoirs. It would be interesting, for example, to try to find similar ^{10}Be peaks at the appropriate depths in deep Arctic ice cores. High concentrations of ^{10}Be have been reported in late glacial ice at Dye 3, Greenland, but these have been correlated with low δ^{18}O values, and thus attributed as mostly resulting from low precipitation rates[23], although the authors did allow for possible contributions due to production or atmospheric circulation effects. We are also planning to investigate various lower-latitude high-deposition-rate marine and lacustrine sediment cores.

Regardless of the eventual source of these ^{10}Be variations, one potential application already seems assured, and that is their use as stratigraphic markers. At the very least, as shown by the evidence from the two cores studied here, these ^{10}Be events should permit correlation of deep ice over a substantial fraction of Antarctica. This could be important for locations for which meteorological or geographical conditions make correlation of oxygen isotope records ambiguous. If the ^{10}Be increases really do reflect production changes, then their presence in other reservoirs would permit a chronological connection between polar and lower latitude climatological records, as well as between the various lower latitude records themselves.

It is also obvious that any inferred change in production of ^{10}Be would have important implications regarding application of other cosmogenic isotopes, such as ^{14}C.

Finally we note that, since the widths of the two ^{10}Be peaks observed here is comparable to our sampling interval (~25 m) over most of the Vostok core, it is not impossible that there are additional peaks of similar or shorter duration, which we have missed. We thus hope to have the opportunity in the future of measuring an even higher resolution ^{10}Be profile in this core.

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Interpreting the anholonomy of coiled light

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Circular birefringence of purely geometric origin was recently predicted[1] and observed[2] in helically coiled monomode optical fibres, and widely reported[3–5] as a successful application to photons of a general theory[6,7] for phase shifts in adiabatically transported quantum states. However, earlier similar observations[8–10] had been interpreted not by quantum mechanics but simply as a classical anholonomy, namely parallel transport of the polarization[11]. Indeed, because the magnitude of the effect is independent of the wavelength of the light as well as Planck's constant, it might seem that 'classical' here means that not only quantum but also wave effects can be neglected. Here, I argue that these experiments, and their discrete analogues, are most appropriately described at the level of classical electromagnetism; the parallel transport law can then be derived (rather than assumed[8–11]) and nonadiabatic polarization changes calculated.

In the quantum description[1], photons in right- or left-circularly polarized light are assumed to be in the eigenstate of positive or negative helicity defined by the local tangent vector **t**(s) of the fibre (Fig. 1). Because the input and output ends of the fibre are parallel, the eigenstate is transported round a closed loop in **t** space and thereby acquires the geometrical phase shift appropriate to spin one, namely[6] minus or plus the solid angle Ω subtended by the loop at the origin of **t** space. These opposite phase shifts $\mp\Omega$ imply[1] that the direction of linear polarization will be rotated by Ω, and it is this gyrotropy that was observed[2,8–10].

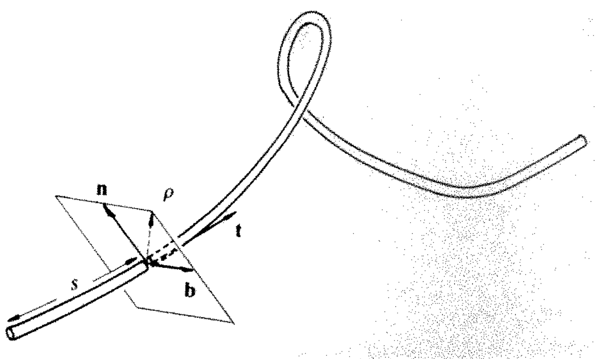

Fig. 1 Geometry of helically coiled optical fibre.

Underlying this successful prediction there are, however, several uncertainties. In the absence of any obvious governing Hamiltonian it is not clear why the phase continuation rule of the general theory[6] is applicable. Even if it is, the fact that the two helicity states are degenerate suggests that the application could equally be made to any superposition of them, yielding different results. (Relativistic arguments[12] do yield the phase continuation rule, and also uniquely select the helicity states, but are inapplicable within fibres.) Moreover, it is hard to see how this type of quantum description can provide estimates of the probability that the coiling will produce nonadiabatic transitions to the opposite helicity. In any case, the high photon flux in all the experiments so far carried out makes a quantum description unnecessary.

At the level of geometrical optics (shortwave limit) it is known[13,14] that in a medium with smoothly-varying refractive index μ the electromagnetic field vectors are parallel-transported along a light ray. This result should, however, not be invoked to explain experiments with monomode fibres, because their fields cannot legitimately be described by ray optics.

In classical electromagnetism the field is governed by Maxwell's equations, with μ depending only on perpendicular distance ρ from the fibre axis. Using the ideas of coupled local modes and the weak-guidance approximation[15], we can write the transverse electric field **E** at position s along the fibre as a superposition of fields linearly polarized along the fibre normal $\mathbf{n}(s)$ and binormal $\mathbf{b}(s)$[16]; this is an adiabatic approximation (with s playing the role that time does in quantum mechanics), valid for gently coiled fibres. Thus,

$$\mathbf{E}(\rho, s) = \exp\{i\beta s\} f(\rho)[c_1(s)\mathbf{n}(s) + c_2(s)\mathbf{b}(s)] \qquad (1)$$

where β and $f(\rho)$ are, respectively, the propagation constant and modal amplitude appropriate to the straight fibre. Substitution into Maxwell's equation gives, after some analysis (to be published elsewhere), the following effective Hamiltonian evolution equation for the polarization coefficients c_1 and c_2 in terms of the fibre curvature κ and torsion τ:

$$i\frac{\partial}{\partial s}\begin{pmatrix} c_1(s) \\ c_2(s) \end{pmatrix} = \begin{pmatrix} \kappa^2(s)/2\beta & i\tau(s) \\ -i\tau(s) & 0 \end{pmatrix}\begin{pmatrix} c_1(s) \\ c_2(s) \end{pmatrix} \qquad (2)$$

This simplest possible theory neglects radiative leaking, coupling to reflected modes and all elasto-optic effects.

Now $\beta \approx 2\pi/\lambda$ where λ is the light wavelength in the fibre cladding, and κ^{-1} and τ^{-1} are both comparable with the fibre bend distances, so in lowest approximation the term $\kappa^2/2\beta$ is negligible and the torsion terms dominate. Then, for initial linear polarization in direction α (relative to **n**), (2) gives

$$c_2(s)/c_1(s) = \tan\left(\alpha - \int_{-\infty}^{s} ds'(\tau(s')\right) \qquad (3)$$

This is precisely the parallel transport law for **E**, giving, after one complete helical turn, a polarization rotation equal to the

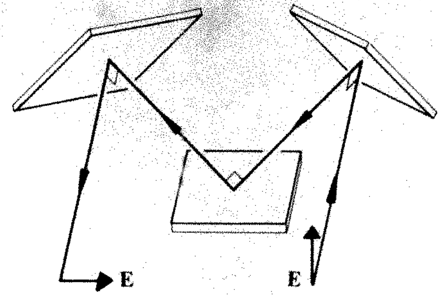

Fig. 2 Antiadiabatic rotation of polarization **E** by three reflections in metal mirrors.

solid angle[10]

$$\Omega = 2\pi - \int_{-\infty}^{\infty} \mathrm{d}s'\tau(s')$$

Moreover, (2) resolves the degeneracy in favour of the helicity states: local eigenmodes (again neglecting $\kappa^2/2\beta$) are circularly polarized, that is, $c_2/c_1 = \mp i$, and acquire opposite phase shifts $\mp\Omega$ round the turn.

Nonadiabatic transitions are induced by the curvature term $\kappa^2/2\beta$. In lowest order this gives the probability for a change from (say) + to −, as

$$P_{\mp} = \left| \int_{-\infty}^{\infty} \mathrm{d}s\kappa^2(s) \exp\left\{2i \int_{-\infty}^{s} \mathrm{d}s'\tau(s')\right\} \right|^2 \bigg/ 16\beta^2 \qquad (4)$$

For a helix uniformly wound on a cylinder of radius R so as to produce phase shifts $\mp\Omega$, this can be written as

$$P_{+-} = [\Omega/2\pi(2-\Omega/2\pi)]^3 \sin^2\Omega/(1-\Omega/2\pi)^2/16\beta^2R^2 \qquad (5)$$

Even for $R \sim 1$ mm this never exceeds 10^{-8}. For planar bends ($\tau = 0$) there is no polarization rotation, and the eigenmodes of (2) are linearly polarized with a tiny bend-induced shift $\kappa^2/2\beta$ in the local propagation constant of the mode polarized along **n**.

It has been suggested (J. N. Ross, personal communication and ref. 17) that the coiling of **t** can be accomplished by (at least three) discrete reflections, the resulting sudden changes in **t** being simulations of adiabatic change. But ideal mirrors (infinite conductivity) do not conserve helicity; they reverse it, and with this 'antiadiabaticity' the solid angle Ω must be accumulated with **t** replaced by −**t** on alternate segments of the light path[17]. If the light beam is reversed after three successive reflections, each changing its direction by 90° (Fig. 2), the predicted polarization rotation (see also ref. 18, pages 84–86) is also 90°. Real metal mirrors (finite conductivity) do not quite reverse helicity: the 'nonadiabatic' probability for preserving the original helicity is

$$P_{++} \approx 1/(2|\mu|^2) \qquad (6)$$

which for silver with $\mu = 0.2 + 3.44i$ is 0.042.

The simulation of true adiabatic change with discrete reflections can be achieved only by total internal reflection in a dielectric with index μ at the critical incidence angle $i = i_c = \sin^{-1}(\mu^{-1})$ (this follows from Fresnel's formulae[13]). For glass, $i_c \approx 45°$, so that rotation of 90° can be accomplished by successive reflections in three 45° right prisms arranged as in Fig. 3. However, i_c is not precisely 45° (because $\mu \neq \sqrt{2}$), and this will cause nonadiabatic helicity switching, with probability

$$P_{+-} = (\mu^2\sin^2 i - 1)/(\mu^2\tan^2 i - 1) \quad (\sin i > \mu^{-1}) \qquad (7)$$

For $i = 45°$ and glass with $\mu = 1.5$, $P_{+-} = 0.1$.

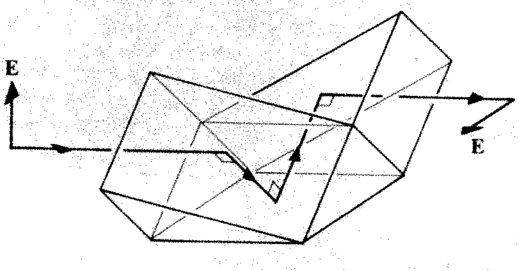

Fig. 3 Adiabatic rotation of polarization **E** by three internal reflections at the critical angle.

The arrangements in Figs 2 and 3 form the basis of robust, simple and (nonadiabatic conditions notwithstanding) convincing lecture demonstrations of the anholonomic transport of polarization.

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Evidence of recent lead pollution in deep north-east Atlantic sediments

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Today, the global lead emissions into the atmosphere from anthropogenic sources are about 200 times higher than in the past[1] and dominate other lead pathways to the ocean. Exhaust from leaded gasoline is the major source of pollutant lead in the atmosphere[2], and subsequently in the marine environment due to atmospheric deposition by wet and dry removal processes[3,4]. This pollution has already perturbed the biogeochemical cycle of lead in the open ocean[2,3,5–7] especially in the north Atlantic. In surface waters, where lead is introduced mainly in the dissolved state, its residence time is relatively short (less than 5 years), as it is rapidly scavenged by, and transported with, particulate matter[5,7]. The mean residence time of lead in the ocean is estimated to be ~100 yr[5,8]. One can therefore expect to see some pollutant lead in the deep ocean surficial sediments. Here, we report lead concentrations in two short cores from the north-east Atlantic. Lead concentrations in surficial sediments (21.0 and 15.0 p.p.m. in the top cm) are higher than in the uppermost 10 cm of the cores (6.0 and 2.8 p.p.m.), and are likely to be enhanced from anthropogenic sources. The quantity

of lead stored in these surficial sediments (5.7 and 2.5 $\mu g\ cm^{-2}$) is of the same order as the amount of pollutant lead present in the dissolved state in the water column.

In this work, we try to establish the degree of lead pollution in two cores of eastern Atlantic sediments sampled during the Fluxatlante cruise in spring 1985 on the RV *Le Suroit.* The sediments were characterized by $CaCO_3$ content, accumulation rate from ^{14}C records, planktonic *foraminifera* biostratigraphy and ^{210}Pb excess ($^{210}Pb_{xs}$) in surficial sediments; this $^{210}Pb_{xs}$ can originate from atmospheric input or production in the water column[9]. The two stations were selected for their different environmental conditions. Station 7 (46°22′ N, 12°23′ W), located on the Armorican Seamount at 3,650 m depth, is several hundred metres above the abyssal plain and about 250 miles from the nearest continental landmass. The sediment is largely biogenous. Station 8 (47°30′ N, 08°31′ W) is located on the Meriadzeck Plateau at 2,200 m depth, on the continental slope, about 150 miles from the French coast, and is influenced much more by down-slope movement of silicate material.

The "Interface" core system (CNRS, Bordeaux) was used to recover sediment samples. The corer allows simultaneous recovery of three short-barrel (length: 50 cm) cores. The inner core barrel is composed of rigid polyvinyl chloride to minimize metallic contamination of the sediments. Examination of the cores upon recovery aboard ship demonstrated the presence of fragile surface dwellers, small-scale (cm) surface structures and particulate fluff, indicating good recovery of the sediment surface. The cores were immediately frozen aboard ship, and later sectioned in 3- to 10-mm slices using a circular stainless steel saw. The sediment in direct contact with the saw was discarded (~1 mm). The sediment was dried for 48 h at 60 °C and then crushed using an agate mortar and pestle. The crushed sediment was split into two homogeneous samples and transferred to pre-cleaned plastic Petri dishes until acid dissolution and analysis were carried out under clean conditions. Lead was determined by graphite furnace atomic absorption spectrometry and potentiometric stripping voltametry. The two techniques showed good agreement. The precision of these techniques was ±10%; the blanks showed lead concentrations less than 10% of the lowest lead concentration in the samples. The accuracy was checked by analysing lead in NBS standards. Spectrometric measurements of naturally-occurring low-energy γ-rays were made using a high precision Ge detector to determine the activities of ^{210}Pb and ^{226}Ra[10].

The distribution of total Pb at both stations is shown in Fig. 1*a*. The two stations show an enrichment of Pb in surficial sediment (first centimetre). Below this, Pb concentrations in each core are low and show little variation with depth; these Pb background concentrations are 2.8 ± 0.5 p.p.m. at station 7 and 6.0 ± 0.9 p.p.m. at station 8. $^{210}Pb_{xs}$ could only be detected in the first centimetre of both cores indicating the penetration depth due to bioturbation over the last century (about five half-lives of ^{210}Pb).

The main difference between the cores appears in the sedimentation rate: 2.2 and 4.5 $cm\ kyr^{-1}$ for stations 7 and 8. Taking into account a bulk density of 1.45 and 1.54 $g\ cm^{-3}$ in surface cores at stations 7 and 8, and a calcium carbonate content averaging 87% at station 7 and 63% at station 8, we determined for stations 7 and 8 an average flux of 180 and 1450 $\mu g\ cm^{-2}\ yr^{-1}$ respectively of silicate material and of 1,800 and 2,470 $\mu g\ cm^{-2}\ yr^{-1}$ of $CaCO_3$.

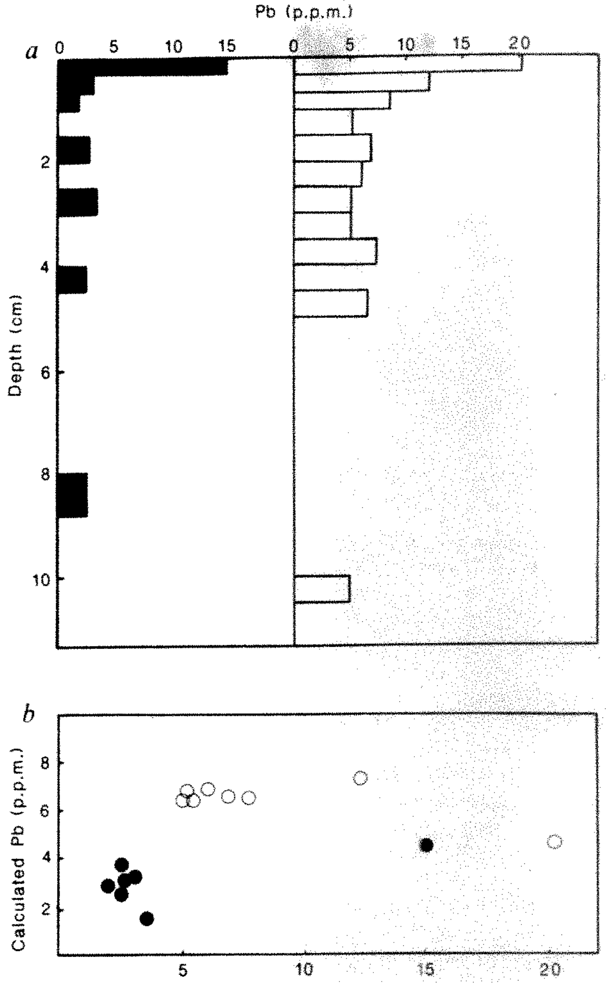

Fig. 1 *a*, Distribution of total Pb ($\mu g\ g^{-1}$ of dry sediment) at station 7 (■) and at station 8 (□). *b*, The calculated concentrations of Pb (using 0.8 p.p.m. Pb in carbonate fraction and 15 p.p.m. Pb in silicate fraction) is plotted against the measured values. The two groups of data represent background Pb concentrations at station 7 (●) and at station 8 (○) respectively. These data can be explained by the relative concentrations of $CaCO_3$ in the two cores. The isolated data represent surface Pb concentrations; they cannot be explained by the calculated data and therefore Pb must have another origin.

First, we consider the background concentration of lead and the reason for a difference of a factor of two in the lead background values between the two cores. Using regressions on each core, we calculated for subsurface samples a theoretical Pb content of 15 ± 3 p.p.m. in the silicate fraction and 0.8 ± 0.6 p.p.m. in the carbonate fraction. These values are consistent with a world average estimate of 12 p.p.m. in the silicate fraction[11] and 0.1 to 1 p.p.m. in the carbonate fraction[12-14]. For subsurface samples, the concentrations of Pb fit satisfactorily those predicted for a mixture of carbonate and silicate material with theoretical Pb contents of 15 and 0.8 p.pm. (Fig. 1*b*). The surficial samples, however, show a significant excess of Pb above the values predicted on this basis (Fig. 1*b*). Considering these results, we can verify that the difference in background lead concentrations between the two stations can indeed be explained by different contributions of carbonate and silicate material in each core (Fig. 1*b*). The background level of lead, as defined here, does not necessarily represent a 'natural' level: indeed, lead pollution has existed for more than 2,000 years. Therefore the background level we refer to here may include some old pollutant lead.

We consider that the Pb concentrations in subsurface sediments correspond to a 'background' level, and we assume that there is no important migration of lead in pore waters: this latter assumption is supported by the fact that isotope ratios of lead in coastal surface sediments differ from subsurface ratios and are close to that observed in the atmospheric input[2].

For our two cores we observe a low organic carbon content (4 $mg\ g^{-1}$ of dry sediment) in the subsurface, characteristic of oxic conditions[15,16]. This observation is confirmed by low concentrations of particulate manganese measured at the interface

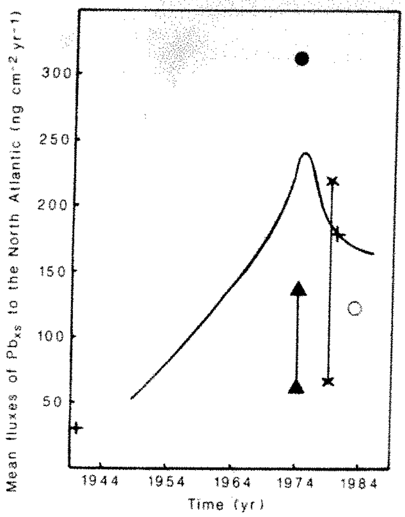

Fig. 2 Mean atmospheric fluxes of pollutant lead (Pb_{xs}) to the north Atlantic. Symbol –, from gasoline exhaust data, see calculation in text, data from Octel, France, and Comité professionnel du Pétrole, Paris, France. Symbol +, (ref. 4); ▲, (ref. 29); ×, (ref. 30); ○, (ref. 22); ●, (ref. 24).

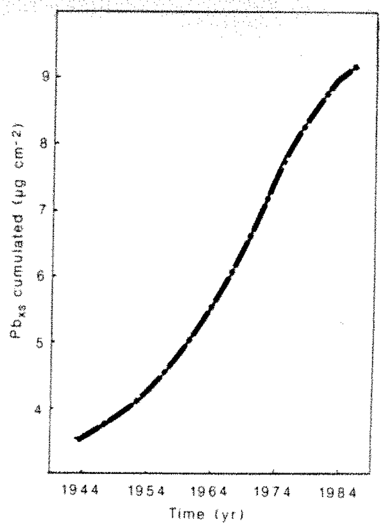

Fig. 3 Average inventory of the input of anthropogenic Pb over the north Atlantic, including Pb accumulated before 1940 (3 µg cm^{-2}, see text) and after 1940 (from Fig. 2).

on a free-carbonate basis (1,000 and 3,160 p.p.m. at stations 7 and 8 respectively). Therefore there is probably little reduction of Mn and Fe oxides in these cores[16] and the excess lead observed in surficial sediments is unlikely to be due to lead migration. Then the excess lead over background (Pb_{xs}) observed in the first centimetre (Fig. 1*a*) could be of recent anthropogenic origin. This is consistent with the fact that the penetration depth of this Pb_{xs} is the same as that of $^{210}Pb_{xs}$ due to bioturbation in the past hundred years.

Another argument for a recent input of anthropogenic lead in surficial sediments is based on existing sediment trap data, although most existing sediment trap material has not been suitable for lead measurements. However, one data set is reported in the Sargasso Sea at 3,200 m depth[17]. It has been shown that the total flux at 3,200 m responds rapidly to surface conditions and that little time elapses between the input of material in surface waters and the flux at great depths[19-22]. The reported deep-sea flux for 1982 (90 ng cm^{-2} yr^{-1}) is in the range of the atmospheric Pb fluxes reported for adjacent waters[22,23], and one can infer that 60-80% of the anthropogenic yearly input in the Sargasso Sea is reaching the sediment.

The Pb_{xs} inventory, taking into account porosity measurements in each core, is 2.5 µg cm^{-2} at station 7 and 5.7 µg cm^{-2} at station 8. At station 7, the silicate flux corresponds to 180 µg cm^{-2} yr^{-1} of silicate material accumulation compared to an open ocean atmospheric input of 50-130 µg cm^{-2} yr^{-1} (refs 24, 25). One can therefore consider that most of the silicate material comes from the atmosphere and infer that most of the lead also comes from the atmosphere which is its main input source. Station 8 is on the slope, closer to the European coast, and subjected to more input from the upper slope, as evidenced by a silicate flux eight times more important at station 8 than at station 7. Geographical variations in the sediment record are to be expected, as it has been shown that Pb is more rapidly scavenged at the boundaries than in the open ocean[3]. How does this inventory of Pb_{xs} compare with the water column inventory and the atmospheric input over the Atlantic?

The dissolved lead of recent anthropogenic origin in the north Atlantic water column can be calculated as equal to Pb concentrations above 5 ng l^{-1} (estimated background concentration). According to the Schaule and Patterson Atlantic profile[26], it amounts to about 3 µg cm^{-2} above the background. This represents 50-100% of the inventory of our cores.

There is little available data on the atmospheric flux of Pb over the north Atlantic. We estimated an average flux for the last 40 yr (Fig. 2) using the data on lead in gasoline (OCTEL) and assuming that two-thirds of the Pb from gasoline exhausts escapes into the atmosphere. The data obtained, from Europe, Canada and USA consumption, were adjusted to the Settle and Patterson Pb atmospheric fluxes for 1981[4]. The other published data from atmospheric lead concentration measurements or estimates are within a factor of two of the calculated average values based on Pb emission data. To calculate an average pre-war industrial lead input over the Atlantic, we used the average 1940 atmospheric deposition flux calculated by Flegal and Patterson[5] and the slope from the data of Murozomi *et al.*[27] showing lead accumulation in Greenland ice from 1750 to 1940. This leads to an average inventory of 3 µg cm^{-2} 'old' industrial Pb before 1940.

Adding both the recent lead input (essentially from gasoline exhaust) and this older industrial input (Fig. 3), we estimate an average accumulated anthropogenic lead inventory of about 9 µg cm^{-2} over the north Atlantic. Considering the inventories of our two cores, ~30-60% of the average input is recorded in the sediment.

Therefore it appears that the two estimates of the north Atlantic atmospheric input from: (1) available data in the atmosphere and (2) Pb inventory in the water column and our results in the sediments are in good agreement. This strongly suggests that a large fration of this pollutant lead is already accumulated in deep north-east Atlantic sediments. If confirmed, this conclusion should be taken into account when quantitatively modelling the future response of the lead cycling in the ocean to changes in the atmospheric input function. Such changes are already occurring due to the phasing out of leaded gasoline in the USA and have recently been recorded in surface marine waters[28].

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Ultra-high resolution ^{29}Si MAS NMR spectra of highly siliceous zeolites

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By preparing the most highly crystalline samples possible and very carefully optimizing all the experimental variables, ultra-high resolution ^{29}Si magic-angle spinning nuclear magnetic resonance spectra have been obtained for completely siliceous zeolite ZSM-5. The spectra show a degree of resolution hitherto not thought possible for rigid crystalline materials, having natural linewidths without any spectral enhancement of ~5 Hz (~0.06 p.p.m.), an improvement of an order of magnitude, and exhibit clearly resolved resonances for 21 of the 24 postulated lattice sites. The resolution is largely maintained with variation of temperature and in the presence of organic sorbates, making it possible to identify clearly the numbers of lattice sites in the resulting new lattice structures.

In our recent investigations of zeolite structures by high-resolution solid state magic-angle spinning nuclear magnetic resonance (MAS NMR), we have found it particularly informative to study completely siliceous analogues formed by hydrothermal dealumination techniques[1,2]. The complete removal of aluminium from the zeolite framework yields very narrow resonances in the ^{29}Si MAS NMR spectrum which are due to the crystallographically inequivalent silicon sites in the lattice structure. Taken together with X-ray diffraction data, the spectra provide a more complete description of zeolite structures, probing such subtle effects in some zeolites as lattice distortions and changes in the lattice structure induced by temperature and by the presence of organic molecules, as well as revealing ambiguities in the spectral determination of the Si/Al ratio of the low Si/Al ratio analogues. In the study of these highly siliceous materials, it is critical that the highest possible resolution be obtained for a particular sample. In this report, we discuss those factors which we have found to be most critical in this regard and present current results for zeolite ZSM-5 under various conditions for use as reference data by other workers in this area. Spectra of comparable resolution may be obtained for other zeolite systems.

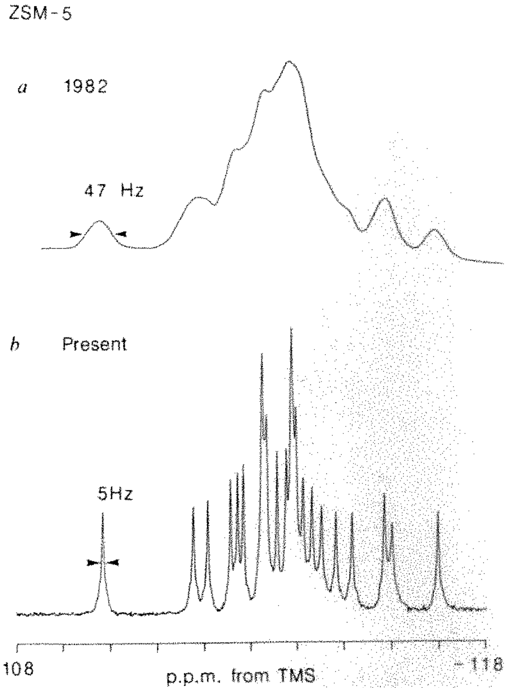

Fig. 1 *a*, ^{29}Si MAS NMR spectrum of highly siliceous ZSM-5 obtained at 79.5 MHz, 6,550 scans, 0 Hz linebroadening. Reproduced by permission from ref. 4. *b*, ^{29}Si MAS NMR spectrum obtained at 79.5 MHz, 1,633 scans, 0 Hz linebroadening (TMS, tetra methyl silane).

We have found it very beneficial to have as intimate a connection between the zeolite syntheses and the NMR spectroscopy as possible. After 'in house' preparation, which permits the deliberate synthesis of very highly crystalline, very high Si/Al ratio materials, the samples are prescreened by XRD measurements and only the very best selected for further treatment. The sample of ZSM-5 used in this present study was prepared by taking four separate solutions A–D consisting of: A, 10 g ammonium bifluoride in 20 ml water; B, 36 Ludox in 123 ml water; C, 6.5 g sodium hydroxide in 10 ml water and D, 10.6 g tetrapropylammonium bromide in 20 ml water. A and B were combined then C and finally D added and the resulting mixture (pH = 8.0–8.5) heated in a stainless steel bomb at 170 °C for 5 days. In the hydrothermal dealumination process, there will be optimum treatment times and temperatures which must be determined. The dealumination conditions used for the present sample were a single continuous treatment at 800 °C for nine days using air saturated with 10% aqueous HCl. The results obtained are for the most part reproducible.

The static magnetic field was shimmed on a liquid sample oriented at the magic angle using the ^{27}Al resonance of $1\ \mathrm{mol\ l^{-1}}$ $\mathrm{Al^{3+}}$ (aq) solution. Homogeneity of ~10 Hz ($\Delta\nu_{1/2}$) could be easily obtained at 400 MHz over the 600 µl sample volume used. Shimming on the ^{2}H resonance of D_2O yields $\Delta\nu_{1/2} \approx 2$–3 Hz. The resulting homogeneity of a spinning sample may be conveniently checked using a spinner filled with a cured polydimethylsiloxane elastomer (a variety of commercial caulking products of this material area readily available). For long accumulations, field stabilization using an external ^{2}H lock may be necessary.

Very-stable-sample spinning was obtained using a modified version of a spinner system by Wind (R. Wind, personal communication). The cylindrical nature of this design also facilitated shimming the magnetic field. Angle adjustment was made by optimizing the sideband intensities of the outer transitions of the ^{81}Br resonance of KBr mixed with the sample as described

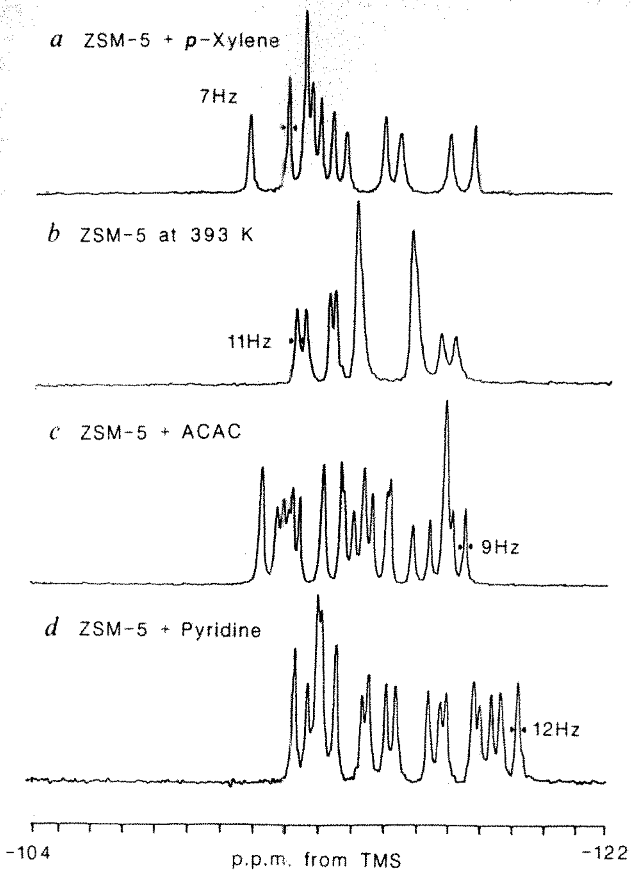

Fig. 2 ^{29}Si MAS NMR spectrum of highly siliceous ZSM-5 obtained at 79.5 MHz. *a*, At 295 K, after the addition of 40 μL of *p*-xylene per gram of zeolite, 500 scans, 0 Hz linebroadening. *b*, At 393 K, 1,225 scans, 0 Hz linebroadening. *c*, At 295 K, after the addition of 80 μL of acetylaceone per gram of zeolite, 4,185 scans, 0 Hz linebroadening. *d*, At 295 K, after the additon of of 140 μl of pyridine per gram of zeolite, 1,122 scans, 0 Hz linebroadening.

by Frye and Maciel[3]. A lever system using a screw thread was used for fine adjustment of the spinning angle with one full rotation of the adjusting rod corresponding to ~1.7 degrees.

The effect of careful optimization of all of the factors discussed above is illustrated in the spectra of the highly siliceous ZSM-5 zeolite in Fig. 1. Figure 1*a* shows the spectrum presented in our original investigation of this system[4]. All of the resonances are due to Si[4Si] environments and reflect the unique local geometries within the ZSM-5 unit cell. The resolution is good enough that the two outer resonances are well defined, allowing the deduction that the unit cell must contain 24 silicon atoms in the basic structural unit. However, only a maximum of nine peaks can be said to be actually observed, although the linewidths are ≈47 Hz (~0.6 p.p.m.) which is very narrow for a rigid solid. Similar spectra have subsequently been obtained by other workers[5-7]. Since then, we have gradually improved the spectral resolution in this system, confirming the relative intensities of the outer resonances in the spectrum[8]. By use of the techniques described above, these improvements have culminated in the spectrum shown in Fig. 1*b* where all of the experimental variables have been carefully optimized. The linewidths have now been reduced to ~5 Hz (0.06 p.p.m.), and provide a degree of resolution hitherto not thought possible for rigid crystalline materials. Twenty-one signals are now clearly observed which can be easily and unambiguously curve resolved. It should be emphasized that no resolution enhancement procedures have been applied to the data presented here. It is anticipated that even further gains in resolution with his sample might be made at higher field strengths and additional increases obtained from the application of resolution enhancement techniques and from alternative data treatments to the fast Fourier transform (FFT) procedure. Experiments in these areas are currently in progress. The main point is that the ultimate resolution obtainable for these very crystalline lattice structures (<0.1 p.p.m.) is very much greater than previously anticipated, making the MAS NMR technique a much more sensitive probe of the lattice structures than was thought to be the case.

The increased resolution is maintained both at elevated temperatures and in the presence of sorbed organic molecules. Thus, on adding *p*-xylene to the system a new spectrum is produced in which 11 lines are clearly resolved (Fig. 2*a*) whose relative intensities indicate 12 independent T sites in the unit cell in agreement with a symmetry change from monoclinic to orthorhombic. Previous spectra resolved only a few of the resonances[9]. In addition, the ultra-high resolution presently obtainable now establishes that the symmetry change is dependent on the organic substrate. There are 24 equivalent T sites in the new lattice structures formed when pyridine and acetylacetone are the sorbates (Fig. 2*c*, *d*).

Greatly improved resolution is also obtained at elevated temperatures although it is somewhat worse than at ambient. The limiting spectrum at 120 °C (393 K) (Fig. 2*b*) is similar in general form to those previously obtained above the phase transition[1,2,10,22] but now some ten resonances are detectable whose relative intensities clearly indicate a twelve tetrahedron basic building unit, again compatible with orthorhombic or pseudo-orthorhombic symmetry, resolving an ambiguity in previous investigations[11]. From spectra obtained at different temperatures, all twelve resonances can be identified.

The phase behaviour of the ZSM-5 lattice under the combined effects of temperature and sorbed organic molecules is exceedingly complex and the ultra-high resolution ^{23}Si MAS NMR spectra which we can now obtain reflect the lattice changes unambiguously and should be invaluable in probing these changes. We hope to improve the quality of the X-ray diffraction determined lattice structures of these systems by using synchrotron radiation and feel that a clear understanding of the phenomenon will result from the combined use of improved data from these two complementary techniques.

It should be possible to attain this degree of resolution for very highly siliceous zeolites in general and we have achieved this for several other systems to date. The present spectra are presented for use as benchmark references in studies of these systems by other workers in the area.

A further implication of these results is that it should be possible to obtain similar improvements in the resolution of the ^{13}C spectra of many crystalline organic materials although local susceptibilities of π-bonded carbons will still limit the linewidths of some resonances. The difference between the present MAS experiments and the conventional CP/MAS (CP, cross polarization) technique is that proton decoupling is not necessary, suggesting that the current limit of resolution in the CP/MAS experiment for very crystalline solids may be the efficiency of the proton dipolar decoupling. Experiments to investigate this possibility are currently in progress.

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Cerium isotope geochemistry of ocean island basalts

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The source of ocean island basalts is one of geology's major problems, and many different models have been proposed. One of these involves recycling of continental and oceanic crustal material into the mantle source of ocean island basalt magmas[1,2]. Island arc basalts are an important link in the chain of evidence that supports this model, as they display isotope signatures in common with both ocean-floor sediment[2,4] and ocean island basalts[1,5]. But hafnium isotope data[6] appear either to place severe restrictions on the quantity of sedimentary material recycled into the mantle, or to demand very thorough homogenization of this material during the subduction process. The lanthanum–cerium isotope method[7] offers a prospect of applying similar constraints to the recycling of different sediment types into the mantle, based on cerium and neodymium isotope systematics. Here I report cerium isotope ratios for eight ocean island basalts. When plotted against published neodymium isotope data these form a 'mantle array' of compositions which indicates coherent behaviour of the two elements during mantle differentiation. This precludes the recycling into the deep mantle of ocean-floor sediments with a large net cerium anomaly.

Cerium undergoes fractionation in the marine environment relative to the other rare-earth elements, such that it undergoes relative depletion in deep ocean water and pelagic sediment, but enrichment in ocean-floor manganese nodules[8]. This fractionation is attributed to the removal of dissolved trivalent cerium by oxidation to a highly insoluble tetravalent state, not observed for other rare-earth elements.

Basaltic lavas from the Mariana Arc display negative cerium anomalies of ~20% relative to other light rare-earths[9], suggesting that cerium-anomalous sediment is subducted into the mantle. If such material were carried down towards the lower mantle it might subsequently be sampled by ocean island basalt (OIB) magmatism.

Roden *et al.*[10] reported a negative cerium anomaly in a tholeiitic basalt from the Koolau series volcanics of Hawaii, and similar anomalies have subsequently been found in East Molokai volcanics[11] and West Molokai volcanics (D. A. Clague and F. A. Frey, unpublished data). If these anomalies reflect ancient material subducted into the OIB source then the greater La/Ce ratios in these samples would have generated higher ^{138}Ce/^{136}Ce isotope ratios than in samples with normal rare-earth element profiles, due to the decay of ^{138}La to ^{138}Ce over geological time.

Lead isotope data yield an apparent age for the mantle source of Hawaiian volcanism of ~10^9 yr[24]. Given the magnitude of the elemental cerium anomalies in Molokai volcanics (>50% in sample 74-WMOL-7; F. A. Frey, personal communication), this time span would be adequate to generate cerium isotopic anomalies very well outside analytical error (>6σ) if the anomaly were the product of ancient recycling of sediment into the mantle. To test this possibility, the anomalous West Molokai basalt sample 74-WMOL-7 was analysed for ^{138}Ce/^{136}Ce, along with a 'normal' Hawaiian basalt and samples from St Helena, the Azores, Samoa, Kerguelen and Tristan da Cunha, which span the range of ^{143}Nd/^{144}Nd isotope ratios in OIB.

Table 1 Cerium isotope data

Sample	Locality	^{138}Ce/^{136}Ce	2σ	N	ε_{Ce}
74-WMOL-7	W. Molokai	1.32828	±6	1,200	−2.2
KL-2	Kiluaea, E. Rift, 1983	1.32829	±8	2,000	−2.1
2882 (NMNH 99658)	St Helena	1.32845	±9	1,200	
2926 (NMNH 99653)	St Helena	1.32844	±9	1,800	
	St Helena average	1.32844	±6	3,000	−1.0
F-33	Faial, Azores, 1958	1.32845	±8	2,700	−0.9
UPO-7	Upolu Puapua, Samoa	1.32857	±7	2,100	0.0
KG14-3	Courbet, Kerguelen	1.32856	±3	1,400	−0.1
TR-1 (NMNH 110014)	Tristan da Cunha, 1961?	1.32862	±7	4,500	+0.4

σ, Standard error; N, number of scans of mass spectrum; ε_{Ce} = parts per ten thousand deviation from bulk-earth composition calculated here.

The analytical techniques adopted here for cerium isotope analysis were similar to those used by Tanaka and Masuda[7]. After separation from major elements and barium on a cation column eluted with HNO_3, very-high-purity cerium separations from the bulk rare-earth elements were made on a 30-cm-long cation column eluted with 0.25 M hydroxy-isobuteric acid (HIBA) at a pH of 4.6. Samples were cleaned to remove HIBA on a miniature cation column. Total blanks averaged 0.4 ng Ce, using reagent quantities appropriate for 0.4 g rock.

Cerium samples were loaded on tantalum-side, rhenium-centre triple filaments, and run as the oxide species on a Vg 54E mass spectrometer at East Kilbride using a single Faraday collector. Following Tanaka and Masuda[7], the ^{140}Ce beam was usually ignored, being 'off-scale' of the amplifier, and analyses were normalized for fractionation to a ^{142}Ce/^{136}Ce ratio of 58.14. A matrix solution for interferences[12] was not considered necessary because BaO- and BaF-induced drifts in ^{138}Ce abundance always stopped before completion of the first 100 sets of peak scans, which were never included in the final analysis. La, Pr, Nd and Sm interferences were always monitored at first, but usually decreased to negligible levels after the first 100 scans. Interference by the peak tail of the very large ^{140}Ce^{16}O beam was accurately corrected using a background shape interpolation, based on measurements in several different positions.

Standards run entirely at low beam current gave an average value of 464.60±0.06 for the non-radiogenic ^{140}Ce/^{136}Ce ratio over a period of several months. This compares well with the value of 464.65 which may be calculated from Tanaka and Masuda[7]. Abundances of ^{138}Ce are normalized here to ^{136}Ce, as this yields results that are much easier to appraise than ^{138}Ce/^{142}Ce ratios.

To be sure of objectively excluding occasional data at the end of long runs when the oxide mass-fractionation law appeared to have broken down, and to check for any long-term undetected interferences, the complete run was analysed in terms of 'super-blocks' of 100 scans of the peaks. Commonly, over 20 such 'super-blocks' were available, usually from two or three separate runs, each of ~8-10 h data acquisition time. Apart from occasional deteriorations in the ratios from dying ion beams at the ends of runs, no long-term drifts in the data were observed. Quoted errors on the analyses are two standard errors on the means of the 'super-blocks'.

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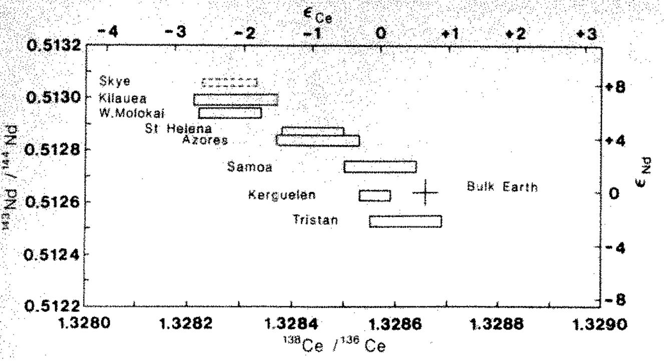

Fig. 1 Plot of $^{143}Nd/^{144}Nd$ against $^{138}Ce/^{136}Ce$ for ocean island basalts using new cerium isotope data and published neodymium isotope data[1,13,14], along with unpublished neodymium data for the W. Molokai sample (D. A. Clague and F. A. Frey). Bulk-Earth point from ref. 15 for comparison.

Cerium isotope ratios (Table 1) are plotted against published $^{143}Nd/^{144}Nd$ data[1,13,14] in Fig. 1. These results define a 'mantle array' of cerium and neodymium isotopic compositions which indicates largely coherent fractionation between cerium and neodymium during mantle evolution processes. The West Molokai sample lies slightly to the left of the Ce-Nd isotope mantle array in Fig. 1, the opposite side to that predicted for incorporation of ancient subducted sediments with a negative cerium anomaly. The elemental anomaly must therefore be comparatively young. The fresh nature of the West Molokai samples rules out sub-solidus alteration, which in any case has not been observed to cause cerium anomalies in any other basalts. However, two other models cannot be easily distinguished. Clague and Frey[11] attributed the elemental cerium anomalies in West Molokai basalts to an unusual oxidation state in the mantle source, but they might also be attributed to the assimilation of young cerium-anomalous pelagic sediment at the base of the volcanic edifice.

A bulk-Earth $^{138}Ce/^{136}Ce$ ratio of 1.32857 is calculated from the Ce-Nd mantle array in Fig. 1. This falls within error of the bulk-Earth point calculated by Shimizu *et al.*[15] from chondritic meteorites, indicating surprisingly good agreement between the East Kilbride and Japanese cerium mass spectrometry, considering the scope for systematic errors in cerium isotope measurement (for example, amplifier non-linearity for very large isotope ratios). But in view of the possibility of systematic offsets in the data, ε_{Ce} values represent the best method for comparison of cerium isotope data from different laboratories, and these are listed in Table 1.

Uncontaminated flood basalts from the British Tertiary Volcanic Province have strontium and neodymium isotopic compositions on the 'mantle array'[16], near the field for Reykjanes Ridge basalts[13]. Therefore, in order to see whether they also fit the Ce-Nd mantle array observed here, an ε_{Ce} value has been calculated from isotopic data on a lava from Skye[17], using a bulk-Earth La/Ce ratio of 0.38 (ref. 18) and lanthanum β-decay constant of 2.3×10^{-12} yr^{-1} (refs 19, 20). This sample falls on the mantle array in Fig. 1, which also attests to the overall coherency between cerium and neodymium fractionation during mantle differentiation.

Patchett *et al.*[6] calculated that up to 1% recycling of pelagic sediment into the OIB source (2% total sediment) would be required if this were the sole process for enrichment of this mantle reservoir. If the pelagic component consisted of material similar to "Pacific authigenic weighted mean sediment"[9], it should (based on calculations in ref. 9, Fig. 9) generate ~50% negative cerium anomalies in the OIB source, which, after storage for 1.5 Gyr would generate cerium isotope anomalies several times the size of the analytical errors achieved here. Hence both cerium elemental and isotopic compositions preclude this model.

Nevertheless, the relatively widespread occurrence of cerium-anomalous basalts in island arcs, including the Lesser Antilles, New Guinea-New Britain, Tonga and Mariana-Izu arcs[9,21-23], suggests that quite substantial quantities of cerium-anomalous material may enter subduction zones. If such material subsequently enters the OIB source, but does not generate net cerium anomalies, it could be balanced by subduction of cerium-enriched manganese nodules. This would imply a uniform mixture of nodule and sediment subduction into the mantle.

Alternatively, cerium-anomalous sediment may enter subduction zones in significant quantities, but be so effectively extracted by partial melting processes below island arcs that very little reaches the deep mantle. This would imply that sediment recycling was not the principal mechanism of trace element enrichment of the OIB source.

Finally, cerium anomalies in island arc basalts might result only from the effect of subducted water on the redox conditions in the mantle melting zone. In this case the anomalies have no causal relationship with the composition of material subducted into the mantle, and no deductions about sediment recycling can be made.

More work must be done to distinguish between these alternative models in detail, but the cerium and neodymium isotope compositions of ocean island basalts demonstrate that on a broad scale these elements behave coherently during mantle differentiation.

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Early agriculture and early Postclassic Maya occupation in western Honduras

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Evidence of the origins, intensification and end of cultivation can be obtained from pollen profiles indicating forest clearance. Independent archaeological data[1,2] (the first occurrences of ceramics and village settlement) have yielded a date of ~3,000 yr BP for the onset of agriculture in western Honduras. Recent research on early Postclassic Maya demography[3,4] has identified much regional variation in settlement densities, contrary to an earlier view, based on limited data, of immediate pan-southern Maya abandonment or depopulation after ~1,050 yr BP (ref. 5). Here I present pollen profiles which provide evidence of early slash-and-burn maize agriculture at Lake Yojoa, probably part of an Archaic hunting and gathering strategy, from ~4,500 yr BP or earlier. Slash-and-burn agriculture intensified at ~3,000 yr BP at Lake Yojoa, and stabilized thereafter. At Petapilla Swamp, 5 km north-east of the major Maya centre of Copan, the pollen sequence began at ~1,000 yr BP and suggests terminal Classic–early Postclassic (AD 950–1200) agricultural activity. The present pollen evidence of occupation until ~AD 1200 and subsequent abandonment of the Copan region reinforces recent archaeological settlement data[6].

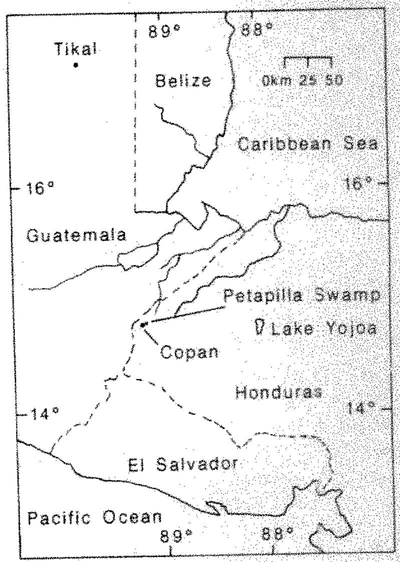

Fig. 1 Index map of sites mentioned in text.

Western Honduras (Fig. 1) is on the southeastern periphery of the southern Maya lowlands, and is transitional to highlands in terrain, climate, and vegetation. Around the Copan Valley (610 m, 1,300 mm annual rainfall) and Yojoa Lake basin (635 m, 2,000 mm annual rainfall) rugged hills rise 1,300–2,000 m. Natural vegetation consists of mesic deciduous tropical forest on valley bottoms, upland deciduous forest on lower hills, and pine-oak montane forest on higher ridges[7], all heavily disturbed by human occupation at present. Agricultural intensification is viable on alluvial soils, but causes erosion and nutrient depletion on slopes.

The present study of two cores collected in 1984 represents the first radiocarbon-dated (uncorrected) pollen sequences from Honduras (Fig. 2*a*,*b*)[8]. A 150 cm core was extracted from the southern shore of Lake Yojoa (18 km north–south, 10 km east–west, 635 m) in 50 cm of water. The gyttja sediments are increasingly coarse below 100 cm. Burned wood fragments from 145 cm yielded a radiocarbon date of 4,770±385 yr BP (sample UGa-5380). The presence of this wood for radiocarbon analysis minimizes hard water error encountered in dating cores elsewhere in Central America[9]. Petapilla Swamp (700 m) is a ~2 × 10^4 m^2 peat bog in an intermontane basin north of the Copan Valley floor. A floating mat of vegetation (Cyperaceae, Ferns, and *Typha*) covers 2 m of water. A 135 cm core of peaty clay was extracted from shallow water at the bog's edge. Radiocarbon analysis of peat from the core yielded two dates: 595±70 yr BP (sample UGa-3579) at 70 cm and 940±60 yr BP (sample Beta-12789) at 130 cm.

Preservation of extant pollen is good in both cores, but there is less diversity in the palynoflora, and even lower representation of tropical deciduous forest trees than in previously reported Central American core sequences[9–11]. Most such trees are insect-pollinated and contribute few pollen grains to sediments. There is no palynological evidence for any significant late Holocene climatic change in the present or previous Central American sequences[9–11], thus allowing the assumption that all vegetational changes are human-induced.

Pollen data are presented as percentages (Fig. 2*a*,*b*). The AP/NAP curve compares all arboreal to non-arboreal pollen.

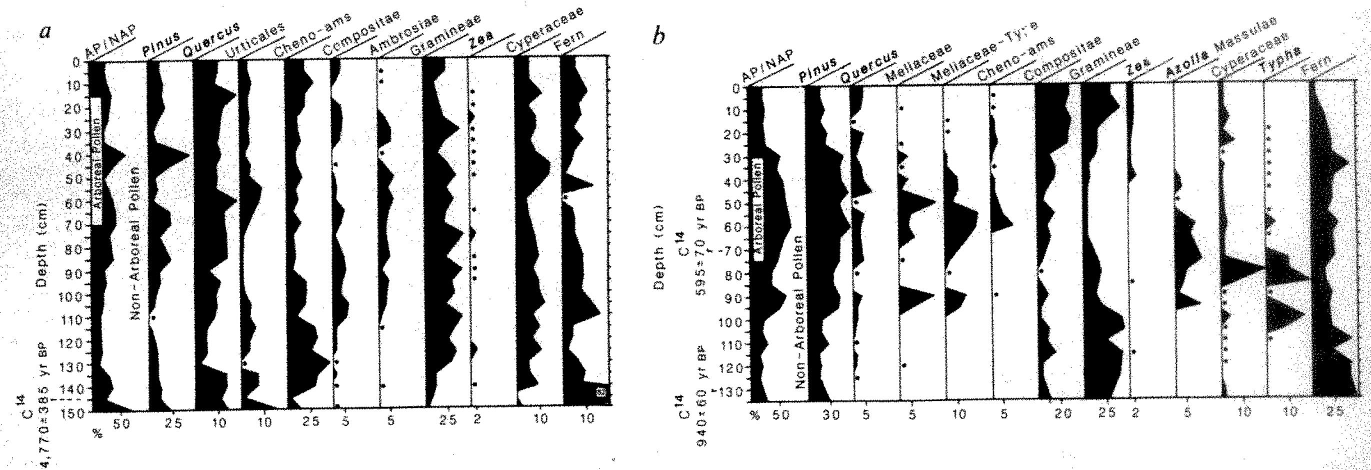

Fig. 2 *a*, A pollen diagram from Lake Yojoa, western Honduras. Analysis made at 5 cm intervals throughout. Percentages based on a pollen sum (greater than 200 grains per level) of total pollen. Dots indicate values of one per cent or less. *b*, A pollen diagram from Petapilla Swamp, western Honduras. Analysis made at 5 cm intervals throughout. Percentages based on a pollen sum (greater than 200 grains per level) of total pollen minus *Pinus* for all curves except AP/NAP and *Pinus*, which are based on total pollen. Dots indicate values of one per cent or less.

Pinus and *Quercus* are dominant members of the montane forest. Urticales, in the Yojoa profile, is an order in which identification of pollen to genus or even family is often difficult. It includes many climax (for example, *Ficus* and *Brosimum*) and successional (for example, *Trema* and *Cecropia*) tropical deciduous forest genera. Meliaceae and Meliaceae-Type, in the Petapilla sequence, are climax deciduous trees in the Mahogany family. Chenopodiaceae-Amaranthaceae ('Cheno-ams'), Ambrosiae, other Compositae, *Zea*, and other Gramineae are herbaceous taxa indicative of human disturbance. *Azolla* (an aquatic fern), Cyperaceae, and *Typha* are hydrophytes. 'Ferns' includes all Filicales.

In the Yojoa sequence (Fig. 2*a*), the zone from 115-150 cm relates to a period of very early slash-and-burn agriculture. Cheno-ams are dominant among herbaceous taxa, and *Zea* is present, although other Gramineae pollen percentages are low, and Ambrosiae and other Compositae are absent. Arboreal percentages decrease from the lowest levels. Disturbance is consistent with that which would be produced by long fallow slash-and-burn agriculture. Because no ceramics or village sites have been identified in Honduras before ~3,000 yr BP[1,2], this early clearance (~4,500 yr BP) probably occurred as part of the expanding food procurement system of preceramic late Archaic peoples, about whom little is known archaeologically[12,13].

Cultivation by nonceramic, principally foraging people has been identified from palaeoecological sequences elsewhere[14,15], and there are indications that such cultivation occurred throughout Central America as well. Phytolithic analysis of sediment cores from Panama date early slash-and-burn activities to ~4,800 yr BP[19], and archaeological evidence of Archaic horticulturalists has been identified tentatively in northern Belize[20]. Previous palaeoecological studies of the Maya in which the chronology of early human disturbance was uncertain[9] equate evidence of the earliest agriculture with the sparsely documented Swasey ceramic culture[16]. But as Swasey ceramics occur at a limited number of sites no earlier than 2400 BC, such disturbance was probably brought about by earlier peoples.

Agricultural intensification at Yojoa is evident above 115 cm with increases in pollen of Ambrosiae and other Compositae (field invaders). Above this point, from ~3,000 yr BP to modern times, there are few identifiable major trends. This indicates a high level of ecological stability even through the Classic period, with no evidence of vegetational changes congruent with the ~AD 900 Late Classic collapse. The maximum of *Pinus* at 40 cm, coeval with a depression in Ambrosiae and other Compositae generally, could relate to a short period of abandonment and reforestation, but such a single level occurrence is inconclusive. Populations in the Yojoa region were probably relatively static after early developments, and agriculture probably continued to be relatively extensive in nature[17].

The pollen sequence from Petapilla Swamp (Fig. 2*b*) is short (beginning at ~1,000 yr BP), because extremely hard clays that perhaps pre-date the formation of the swamp prevented further entry of the coring device employed. The zone from 100 to 135 cm, estimated to date from ~1,000 to 750 yr BP, marks terminal Classic–early Postclassic Maya disturbance. Gramineae pollen percentages are high, *Zea* is present, and the deciduous forest trees Meliaceae and Meliaceae-Type are nearly absent. The forest was completely cleared from the valley floor and foothills during this period. Low *Pinus* percentages indicate that even montane forest may have been partially stripped. Reforestation is evident above 100 cm (after ~750 yr BP), as shown by the rise of *Pinus*, Meliaceae and Meliaceae-Type versus Gramineae. The swamp expands during this period, as shown by increased percentages of hydrophytes. Modern disturbance is present above 30 cm, with the reclearance of the deciduous forest and re-emergence of Compositae and Gramineae.

The presence of *Zea* and concurring recent archaeological evidence in the form of obsidian hydration dates[6] suggest that populations remained in the Copan region until ~750 yr BP. This early Postclassic occupation is contrary to previous independent archaeological interpretations based solely on ceramic chronology[18]. No clearly differentiated ceramic style postdates Conerphase ceramics, which were assumed to end with the cessation of monumental construction ~AD 900. Early Postclassic rural populations must have continued to use utilitarian Coner styles after construction ended at the urban centre. As recent studies of other Maya regions have shown, reduced rural populations remained around generally vacant urgan centres at densities which varied regionally[3,4]. The sociopolitical and economic factors[3,4] producing this variation are incompletely understood.

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Use of non-arbitrary acoustic criteria in mate choice by female gray tree frogs

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The evolutionary significance of female choice is controversial[1]. Central to the issue are the mechanisms and criteria used by females to choose among potential sexual partners. For example, females merely responding to conspicuous displays that are uncorrelated with male condition or quality would neither incur costs of assessment nor gain benefits over and above random mating[2]. Here we show that selective phonotaxis by female gray tree frogs depends on non-arbitrary acoustic cues that indicate the male's species and energetic costs of signal production. Furthermore, some female preferences cannot be explained by differences in signal conspicuousness (acoustic energy) alone. Females obviously benefit by rejecting genetically incompatible, heterospecific males, which can be identified solely by pulse rate. Females also select among conspecific males on the basis of call duration, which is correlated with a male's energetic investment in courtship and probably indicates his physical condition and fitness.

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Table 1 Responses of female gray tree frogs in two-stimulus playback experiments with varying call duty-cycle

Test no.	Quality of alternative stimuli	Duty-cycle ratio	*N*	Responses	*P*
1	12-pulse, 900 calls per h vs 12-pulse, 1,800 calls per h	1:2	15	0:11	<0.01
2	12-pulse, 900 calls per h vs 24-pulse, 900 calls per h	1:2	15	0:12	<0.01
3	20-pulse, 1,132 calls per h vs 30-pulse, 1,132 calls per h	1:1.5	24	3:13	<0.05
4	20-pulse, 1,132 calls per h vs 40-pulse, 1,132 calls per h	1:2	11	0:9	<0.01
5	20-pulse, 607 calls per h vs 80-pulse, 607 calls per h	1:4	25	0:8	<0.01

N, number of females (one trial per female per test); pulse rate, 20 pulses per second ($\mathrm{p\,s^{-1}}$) unless otherwise indicated; pulse duration, 25 ms, except for stimuli of 40 $\mathrm{p\,s^{-1}}$, 12.5 ms. Probability in two-tailed binomial test shown as *P*. Each female was tested at 20 °C (±1.5) in a semi-anechoic, temperature-regulated chamber. Speakers (Analog-Digital-Systems 200) were 2 m apart; sound pressure level of alternatives was 85 dB at the release point of the female, midway between the speakers. A response was scored when a female moved to within 10 cm of a speaker, and are shown as no. of responses to the first alternative: no. of responses to the second. For details, see ref. 9.

Table 2 Responses of female gray tree frogs in two-stimulus playback experiments with minimized call duty-cycle differences

Test no.	Quality of alternative stimuli	Duty-cycle ratio	*N*	Responses	*P*
1	18-pulse, 1,000 calls per h vs 12-pulse 1,385 calls per h	1.08:1.00	11	10:0	<0.01
2	18-pulse, 900 calls per h vs 12-pulse, 1,333 calls per h	1.01:1.00	26	9:12	NS
3	18-pulse, 900 calls per h vs 12-pulse, 1,500 calls per h	0.90:1.00	19	8:4	NS
4	24-pulse, 679 calls per h vs 12-pulse, 1,333 calls per h	1.02:1.00	39	29:7	<0.001
5	24-pulse, 638 calls per h vs 12-pulse, 1,268 calls per h	1.01:1.00	9	8:0	<0.01
6	24-pulse, 644 calls per h vs 12-pulse, 1,331 calls per h	0.97:1.00	14	5:5	NS

Symbols and conditions as in Table 1, except NS, not significant.

Table 3 Responses of female gray tree frogs in two-stimulus playback experiments with pulse rate varied

Test no.	Quality of alternative stimuli	Duty-cycle ratio	SPL ratio	*N*	Responses	*P*
1	20 pulses per s, 20-pulse, 840 calls per h vs 40 pulses per s, 40-pulse, 1,560 calls per h	1.00:1.90	1:1	13	12:1	<0.01
2	20 pulses per s, 20-pulse, 840 calls per h at 72 dB vs 40 pulses per s, 40-pulse, 840 calls per h at 90 dB	1.00:1.00	1:8	14	7:0	<0.02

Symbols and conditions as in Table 1, except that pulse rate and sound pressure levels are as shown.

Selective phonotaxis was studied by offering gravid females of *Hyla versicolor* simultaneous choices of 'synthetic sounds'. In the first set of tests, alternatives differed in either call duration or call repetition rate (call rate), but not both. Each pair of stimuli differed by at least a factor of 1.5 in terms of the call duty cycle (number of sound pulses per second of calling) and hence the amount of acoustic energy per unit time. Females invariably preferred the alternative with more sound energy (higher duty-cycle ratio), and even chose signals with combinations of duration and call rate that were more extreme than those observed in nature (Table 1, Fig. 1*a*, trace 3 in Fig. 1*b*). Halliday[3] proposes that responsiveness to such 'supernormal' stimuli may be a mechanism for female choice. However, a supernormal stimulus for the gray tree frog is, by definition, a 'conspicuous' signal with much more than average acoustic energy and hence an example of what Parker[2] terms passive attraction. Another example is the female gray tree frog's attraction to the louder of two calls[4,5].

In the second set of tests we varied both the duration and call rate of the alternatives so that the duty cycles of the stimuli, and hence acoustic energy, differed by 1–10% (Table 2; Fig. 1*b* traces 1 and 2). When duty-cycle differences favoured long calls by 2% or more, females preferred long calls to short calls (tests 1 and 4 in Table 2). When duty cycle favoured short calls, even by as much as 10%, there was no preference for either stimulus (tests 3 and 6 in Table 2). When duty-cycle differences were ~1%, females chose a 24-pulse call over a 12-pulse call but did not prefer an 18-pulse call over a 12-pulse alternative (tests 2 and 5 in Table 2). Thus, females were not simply attracted to the stimulus pattern with more acoustic energy, but were biased toward long calls.

Taigen and Wells[6] show that aerobic metabolism in gray tree frogs is positively correlated with call duration and call rate: their product accounts for about 84% of the variance in aerobic metabolism during calling. Wells and Taigen[7] speculate that production of long calls depletes glycogen reserves more rapidly than production of short calls. Three additional lines of evidence indicate that long calls are more costly to produce than short calls, even when call duty-cycle differences are minimal as in the experiments just described. First, male gray tree frogs usually increase call duration in competitive situations (dense choruses or in response to playbacks of conspecific calls)[7]. Males typically compensate by lowering call rate. Note, for example, that none of the males recorded in Missouri produced calls with both the duration >850 ms and the call rate >1,000 calls $\mathrm{h^{-1}}$ (Fig. 1*a*). Second, call duration is negatively correlated with the time spent calling during an evening[7]. Third, male gray tree frogs give an exceptionally long call when they are touched by a female but do not achieve amplexus with her[4]. Males revert to baseline call duration immediately, suggesting that they cannot sustain the production of extremely long calls (G.M.K. and H.C.G., unpublished data).

So far we have discussed call properties representative of natural variation among conspecific males. In a third set of tests, we varied pulse rate, a species-specific property. Females strongly preferred synthetic calls with a pulse rate typical of conspecifics to alternatives with a higher pulse rate, in the range of a sibling species, *H. chrysoscelis*, which differs in chromosome

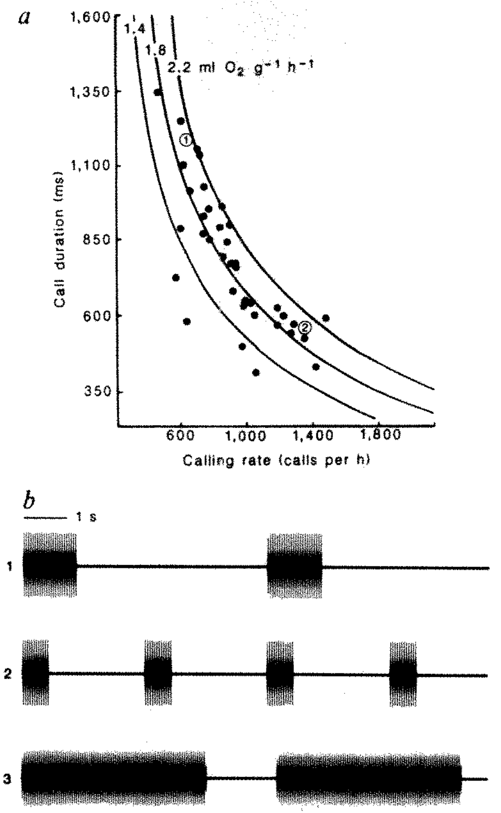

Fig. 1 *a*, Values of call duration and call rate (solid circles, calling effort) of 37 males of *Hyla versicolor* from Boone County, Missouri. Notice that if call duration was long, call rate was low. Thus, aerobic metabolism during calling (shown as solid contour lines, estimated from Wells and Taigen[7]) was usually less than 2.2 ml $O_2 g^{-1} h^{-1}$. In both Missouri and Connecticut[7], males usually increased duration and lowered call rate when challenged by a playback of conspecific calls. *b*, Oscillograms of synthetic sounds used in playback tests of females of *H. versicolor* from Boone County, Missouri. Traces 1 and 2, 24- and 12-pulse calls respectively; trace 3, supernormal stimulus. In traces 1 and 2, the call rate of the short call was about double that of the long call; only a detailed analysis over several minutes reveals that the call duty cycle (number of pulses of 25 ms per second of calling) of the long call was ~1% greater than that of the short call. The calling effort and estimated oxygen consumption during calling that correspond to these two stimuli are indicated in *a* by the open circles. During playbacks to females (Nagra IV-S stereophonic recorder, one stimulus per channel), the timing relationship of the two alternatives was not fixed but rather the sounds drifted in and out of phase during the course of a trial. Control tests showed that the relative timing of two identical signals did not affect female preferences and that the preferences between the two signals illustrated could not be explained by the masking of one stimulus by the other. The third oscillogram illustrates a 'supernormal' stimulus; males never produced such long calls at such a high rate. The alternative to the 'super' long call was a normal 20-pulse call, the timing relationship of which was fixed so that it was always presented during the silent interval between two super long calls.

number. Preferences persisted even when the call rate of the 'conspecific' stimulus was half that of the alternative and, even more remarkably, when its sound pressure level was eight times less (18 dB) (Table 3). Thus, a female would reject a male producing an inappropriate pulse rate, regardless of the acoustic energy of his call relative to that of a conspecific male. This is a clear example of what Parker[2] terms active choice.

What is the evolutionary significance of our results? The (acoustic) energy-independent preference (Table 3) for sounds that differed in pulse rate, a heritable call property[8], almost certainly reflects the fact that females mismating with *H. chrysoscelis* produce sterile hybrids[9]. The existence of such species recognition mechanisms is the starting point of Fisher's[10] theory of sexual selection.

The preferences of the tree frogs for metabolically costly signals, as opposed to apparently arbitrary ones[11,12], is suggestive, but more problematic. Selective phonotaxis for louder, longer, or more rapidly-repeated calls (Table 1 and ref. 4) cannot be distinguished from passive attraction, which Parker[2] thinks is best viewed as a form of male-male competition because females may gain little, if anything, over and above random mating. Andersson[13] argues that female choice for 'good genes' can evolve and be maintained. If exaggerated displays reflect a heritable component of fitness, then females exercising a choice would gain even more than the production of male offspring of greater than average attractiveness to females. We argue that energetically costly calls indicate at least that a mate is in good physical condition. Aerobic metabolism is often higher during calling than during forced exercise[6]. Moreover, the fact that females usually preferred long calls (Table 2), even when stimulus (acoustic) energy was about the same as that of short calls, indicates active choice. We predict that females using this criterion will benefit more than females which mate randomly.

We know of no data concerning heritability of call duration in gray tree frogs nor of fitness in any anuran, but recent evidence from birds[14] indicates that differences in patterns of vocal production may be correlated not only with lifetime reproductive success but also survivorship. Studies of insects have documented the genetic basis of female preferences[15], and frog vocalizations are polygenic traits[16] for which variance is probably maintained in the face of selection[11]. As Majerus[15] points out, there is no example in which the complete system of sexual selection through female choice has been elucidated.

In summary, we have rigorously demonstrated female preferences for qualities of male displays that are not arbitrary, but rather serve to prevent hybridization or indicate directly the energetic investment of a male in courtship. These attributes of the male are encoded by different, independent parameters of the same signal, pulse rate and call duration, respectively. We argue further that the quantification of the strength of a preference, as measured by its intensity-independence, indicates the relative benefit that a female derives from choosing among males.

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Genetic linkage between X-chromosome markers and bipolar affective illness

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A pedigree study shows close linkage of bipolar affective illness (manic depression) to the X-chromosome markers colour blindness and glucose-6-phosphate dehydrogenase deficiency. The maximum lod score ranges from 7.52 (assuming homogeneity) to 9.17 (assuming heterogeneity); that is, the odds in favour of linkage range between 3×10^7 to 1 and 10^9 to 1. These results provide confirmation that a major psychiatric disorder can be caused by a single genetic defect. As a possible first step in characterizing the primary genetic abnormality, this finding may have important implications for the aetiology, nosology, pathophysiology and, possibly, prevention and treatment of bipolar affective disorder. It also provides a means for identifying and characterizing homogeneous populations of patients and may help in clarifying aetiological heterogeneity.

The X-linkage hypothesis of bipolar illness has drawn considerable attention in the psychiatric and genetic literature. The hallmarks of X-linked dominant inheritance, namely, excess of affected females and lack of male-to-male transmission, have been observed in some family studies; however, instances of male-to-male transmission have also been described[1,2].

Linkage studies with the X-chromosome markers colour blindness, glucose-6-phosphate dehydrogenase (G6PD) deficiency and the Xg blood group have yielded conflicting results, with some pedigrees showing evidence for linkage to colour blindness or G6PD deficiency[3-9], and others showing evidence against close linkage[10]. The conflicting data and methodological uncertainties in some of the earlier studies[4,5] have been the subject of scrutiny[11]. We[1,12,13] and others[14] have recently addressed these issues in a reanalysis of the linkage data. The discrepancy among the various data sets was explained by genetic heterogeneity with close linkage of bipolar illness to colour blindness and G6PD deficiency in some pedigrees; close linkage to Xg blood group was ruled out[12].

Although the diverse results can be attributed to genetic heterogeneity, the controversy has engendered calls for independent replication using a new series of pedigrees.

Our pedigrees originated from the patient population of the Jerusalem Mental Health Center. The X-chromosome markers were colour blindness and G6PD deficiency. The loci for these traits have been localized to the distal long arm of the X chromosome (q27-qter and q28 for the colour blindness locus and *G6PD*, respectively) and are known to be closely linked[15,16]. The population selected is particularly suitable because it is characterized by: (1) its relatively high lifetime prevalence of affective disorders generally and bipolar illness in particular[17]; (2) the low population rates of alcoholism and drug abuse, conditions that may confound the psychiatric diagnosis; (3) large, multigenerational pedigrees, especially in the non-Ashkenazi community[18]; (4) stability and accessibility of family members[18]; and (5) high frequency of G6PD deficiency among non-Ashkenazi Jews—as high as 60% among Kurdish males[19].

The screening of probands took place between 1982 and 1985. Criteria for defining probands were: age 16 or over; a confirmed hospital diagnosis of bipolar I affective illness[20,21]; knowledge of biological parents; and consent to participation in the research. Using the family history method, data were obtained from the proband and a first-degree relative considered to be the best informant and were supplemented by medical records. We gave preference to larger families and required that at least one immediate relative be affected with bipolar or a related major affective illness. Families with male-to-male transmission were excluded because the hypothesis of X-linkage assumes X-chromosome transmission. Families that fulfilled these initial criteria were screened for colour blindness and G6PD deficiency by obtaining information on family members from the proband and available relatives. Data on colour blindness and G6PD deficiency collected by the Israeli army on new recruits were also used, when available. Families found to segregate for either marker underwent a more extensive evaluation and these studies were extended to multigeneration pedigrees.

The probands and all available informative adult relatives (over 16) were personally examined using a slightly modified version of the Schedule for Affective Disorders and Schizophrenia (SADS)[18]. Diagnoses were based on the Research Diagnostic Criteria (RDC)[21] using a diagnostic hierarchy[22]. A total of 85% of the relatives were personally interviewed; 5% underwent telephone interviews. Clinical data on unavailable relatives were obtained from their closest relatives using the Family History-Research Diagnostic Criteria (FH-RDC)[23]. The FH-RDC method also served to supplement the personal interviews. Medical records were used when available. The clinical data were gathered by experienced mental health professionals. Good inter-rater reliability was achieved both before and during data collection; the final diagnoses were made by the clinical interviewers using all available information.

All probands met criteria for a diagnosis of bipolar I affective illness. The bipolar-related category in families of bipolar I probands reportedly consists of the following major affective disorders[22,24]: bipolar I, bipolar II, manic (without depression),

Table 1 Lod scores for linkage of affective disorders to various markers

Marker	Pedigree no.	Ethnic origin	Recombination fraction (θ) 0.00	0.05	0.10	0.15	0.20	0.30	0.40
CB	015	NA	2.69	2.37	2.04	1.69	1.32	0.51	−0.21
(Colour blindness locus)	017	NA	2.31	2.11	1.90	1.68	1.45	0.94	0.40
	019	A	−4.19	−1.07	−0.69	−0.46	−0.31	−0.12	−0.02
	037	NA	1.34	1.33	1.27	1.18	1.08	0.78	0.41
Total									
All pedigrees			2.15	4.74	4.52	4.09	3.54	2.11	0.58
A pedigree			−4.19	−1.07	−0.69	−0.46	−0.31	−0.12	−0.02
NA pedigrees			6.34	5.81	5.21	4.55	3.85	2.23	0.60
G6PD	009	NA	2.94	2.67	2.38	2.07	1.75	1.07	0.37

NA, Non-Ashkenazi; A, Ashkenazi.

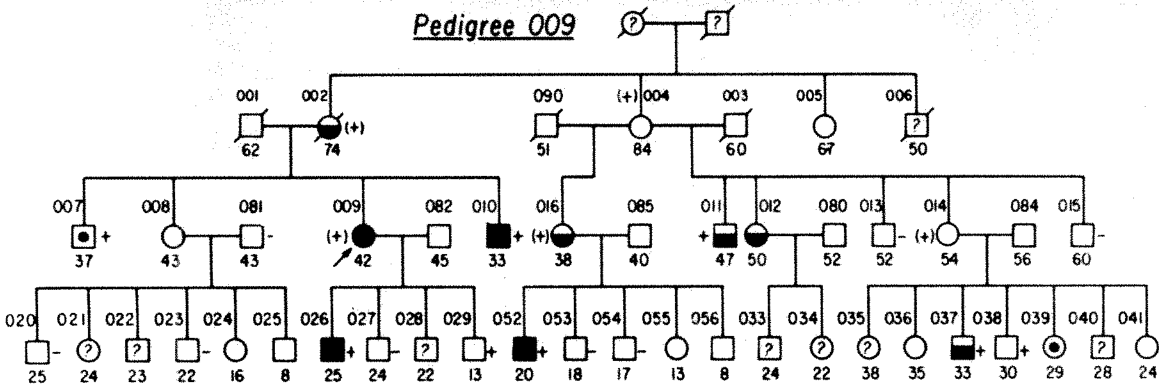

Fig. 1 Pedigree showing affective illness and G6PD deficiency. Symbols: arrow, proband; circles, females; squares, males; a slashed symbol indicates that an individual is deceased; a filled symbol denotes bipolar I, bipolar II, manic or schizoaffective (mainly affective) manic disorder; a half-filled symbol indicates unipolar or schizoaffective (mainly affective) depressive illness; and a centred dot stands for cyclothmic disorder; a blank symbol indicates other diagnoses (mainly normal) unrelated to the bipolar affective spectrum; the number above a symbol is the individual's identification number; the number below a symbol is the individual's age (in deceased individuals the age at death—if known—is indicated); +, −, and (+) denote the presence of the marker (colour blindness or G6PD deficiency), its absence, and a carrier state, respectively; the marker status is unknown where one of these symbols does not appear; ? signifies unknown psychiatric status in an adult relative (age 16 and over); individuals under age 16 were not examined.

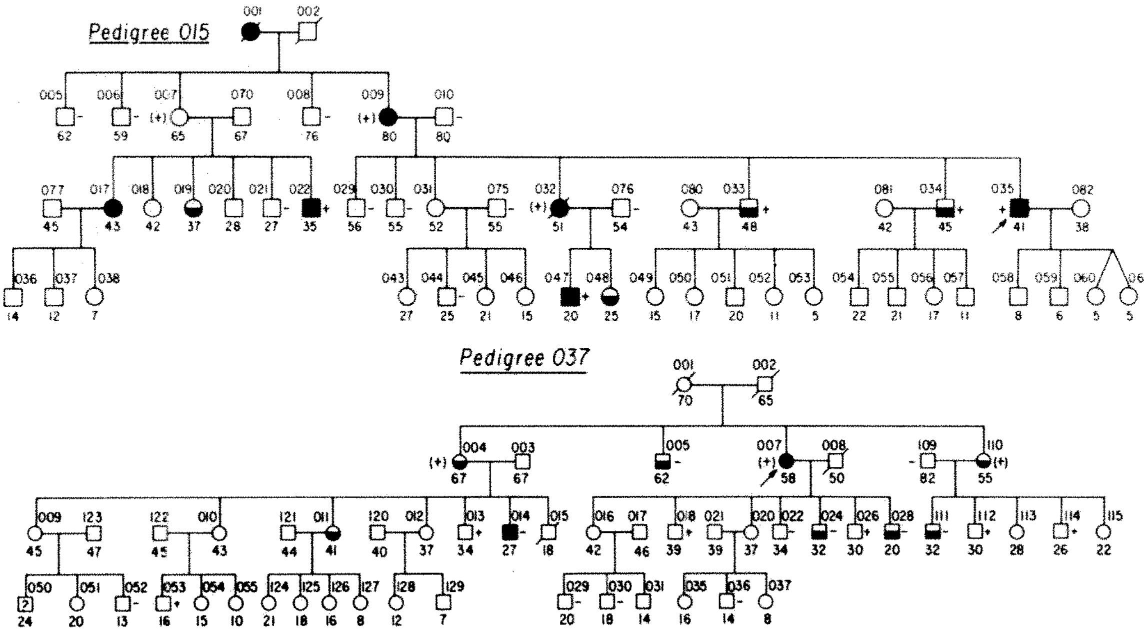

Fig. 2 Pedigrees showing affective illness and protan colour blindness. Symbols as for Fig. 1.

unipolar (major depression without mania or hypomania) and schizoaffective—mainly affective. A milder affective condition called cyclothymic disorder has also been claimed to be part of the bipolar 'spectrum'[22]. In accordance with the cited literature, the 'affected' category among the relatives in our pedigrees was defined by the aforementioned diagnoses.

Colour vision was examined using the Ishihara plates[25]. Psychiatric evaluations were done independently of prior data on colour vision status; these evaluations preceded the colour vision testing in order to minimize the possibility of bias affecting the psychological assessments.

G6PD activity in erythrocytes was determined by the spectrophotometric method[26], the assay being done within three days of blood sampling. Split-sample and test-retest reliabilities were both high. G6PD determination and psychiatric diagnoses were always done blind to one another.

For unavailable relatives, family history for the presence of colour blindness (and the military record, if known) was accepted if there was corroboration from several relatives (one case); however, reports of absence of colour blindness (unless corroborated by the army test results) were discounted and the person's status was considered unknown. Family history on G6PD status was accepted only if corroborated by medical records and/or the army test results (two cases); otherwise, the person's status was considered unknown.

The pedigrees that were found to be informative for the analysis of X-linkage are shown in Figs 1-3. The pedigrees contained 161 adult individuals, 47 of whom were ill with bipolar illness or related affective disorders (bipolar I, 14; bipolar II, 5; manic, 1; unipolar, 20; schizoaffective, mainly affective-manic, 2; schizoaffective, mainly affective-depressed, 2; cyclothymic, 3). There were at least 6 individuals in each pedigree with major affective illness (range: 6-11), 2 or more of whom had bipolar I, bipolar II, manic or schizoaffective-manic disorder (range: 2-8). Consanguinity was present in three pedigrees; the proband's parents in pedigrees 009 and 015 were first cousins by way of the paternal and maternal grandfathers; the proband's parents in pedigree 037 were first cousins by way of the paternal and maternal grandmothers (these portions of the pedigrees are not shown).

Four of the five pedigrees (numbers 009, 015, 017, 037) were non-Ashkenazi, whereas pedigree 019 was Ashkenazi. The non-

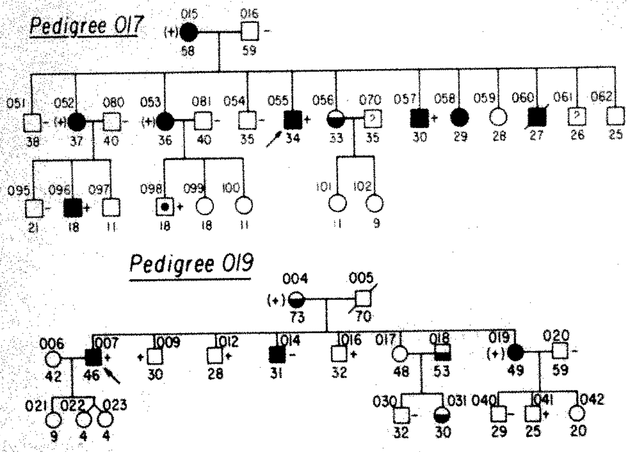

Fig. 3 Pedigrees showing affective illness and deutan colour blindness. Symbols as for Fig. 1.

Ashkenazi pedigrees originated from: Iraq (009), Yemen (015), Turkey (017) and Iran (037). The Ashkenazi pedigree originated from Poland. Pedigrees 015 and 037 segregated for protan and pedigrees 017 and 019 for deutan colour blindness, respectively. Pedigree 009 was informative for G6PD deficiency. The linkage phase in pedigrees 009, 015, 017 and 019 was coupling (that is, the alleles for the two traits—the illness and the marker—were both on the same X chromosome); the linkage phase in pedigree 037 was repulsion (the alleles for the two traits were on different X chromosomes).

The data were analysed for linkage of colour blindness or G6PD deficiency to the putative bipolar disease locus using the computer program LIPED[27], allowing for age-dependent penetrance[28] with a square-root normal age-at-onset function as described[12]. In these data, mean age at onset was 19.6 years, but we used 21 years to be conservative. The data on colour blindness were combined. The frequency of the alleles for bipolar illness, colour blindness and G6PD deficiency was assumed to be 0.01, 0.04 and 0.25, respectively, based on population estimates[19].

The maximum cumulative lod score (the logarithm of odds in favour of linkage) for the colour blindness pedigrees was 4.75 at recombination fraction $\theta = 0.04$; the maximum lod scores for individual pedigrees ranged from 0.00 (pedigree 019 at $\theta = 0.50$) to 2.69 (pedigree 015 at $\theta = 0.00$). The maximum lod score for the *G6PD* pedigree was 2.94 at $\theta = 0.00$. The lod scores are displayed in Table 1 and Fig. 4.

For the analysis of linkage heterogeneity, we used both Morton's likelihood ratio test[29] and an alternative, two-recombination fraction heterogeneity test[12]. With either method, the degree of heterogeneity among the four colour blindness pedigrees was marginally significant: $\chi_3^2 = 7.32$, $P < 0.10$ and $\chi_1^2 = 2.91$, $P < 0.10$, respectively. All the pedigrees that support linkage had a non-Ashkenazi background whereas the only Ashkenazi pedigree had negative lod scores. The maximum lod score in the non-Ashkenazi colour blindness pedigrees is 6.34 at $\theta = 0.00$; in contrast, the corresponding lod score in the Ashkenazi pedigree at $\theta = 0.00$ is -4.19 (Table 1).

Table 2 presents a multipoint analysis for the combined lod scores for both colour blindness and G6PD. Because no pedigrees segregated both markers together, the analysis was easily done by hand. We assume the distance between the colour blindness locus and *G6PD* is 2 map units, or centimorgans, one per cent recombination ($\theta = 0.01$) being equal to 1 centimorgan; it is still unknown as to which marker is distal and which is proximal[15]. We have arbitrarily placed colour blindness at location 0 and represent *G6PD* as to the 'right' of it, with a positive map location (see Table 2). The inference about location of the bipolar locus and the maximum total lod score depend on the assumption of linkage heterogeneity or homogeneity among the colour blindness pedigrees. When we assume heterogeneity (excluding the single Ashkenazi pedigree), the maximum combined lod score occurs when the bipolar locus coincides with the colour blindness locus with a lod score of 9.17, or odds of 10^9 to 1 in favour of linkage. Moving in either direction from the colour blindness locus reduces the odds, for example, at 10 centimorgans on either side the odds have decreased by more than 10-fold. If we assume homogeneity, and include the Ashkenazi pedigree, the maximum lod score occurs when the bipolar locus is placed 5 centimorgans to the right of colour blindness (3 centimorgans to the right of *G6PD*), with a lod score of 7.52, or odds of $3 \times 10^7 : 1$ in favour of linkage. The odds decrease rapidly as the possible location of the affective disorders locus moves towards the colour blindness locus, but then increase again as it moves farther away. The odds drop about 10-fold at 10 centimorgans to the right of the *G6PD* locus and 15 centimorgans to the left of this marker. Hence, the most probable location of the disease locus is close to and possibly between the two marker loci.

We also analysed the data using a narrower definition of the affected phenotype. When the relatives with cyclothymic disorder—individual 007 in pedigree 009 and individual 098 in pedigree 017 were classified as normal, the maximum lod scores for colour blindness and G6PD corresponding to those given above were 4.37 and 2.02. These lod scores are smaller than those obtained using the wide definition of the phenotype, but they nonetheless strongly support linkage.

In our analysis, we have assumed nearly complete penetrance for the X-linked gene at high values of age. We also analysed the data allowing for reduced penetrance and varying the gene frequency. Only small differences were obtained. Hence, the presented results are robust with regard to assumptions about gene frequency and penetrances. The results overall strongly support the existence of a genetic locus which predisposes to bipolar and related affective disorders and is closely linked to the colour blindness and G6PD loci.

This particular linkage in the population was only found in the non-Ashkenazi pedigrees, all of which showed positive lod scores. Although the number of Ashkenazi pedigrees was insufficient for comparative purposes, it is tempting to speculate that X-linked inheritance of bipolar affective illness may be more pronounced among non-Ashkenazi Jews (and, possibly, among other Mediterranean and Asian communities which share part of the same gene pool) than in other ethnic groups. Additional evidence is provided by a previous report on a large Jewish pedigree of Iranian extraction showing strong evidence

Table 2 Lod scores for location of bipolar disorders locus

Map location (centimorgans from *CB*)† [Markers]	−15	−10	−5	0 [*CB*]	+2 [*G6PD*]	+5	+10	+15
Assuming heterogeneity (non-Ashkenazi families only)	6.49	7.47	8.36	9.17*	9.07	8.55	7.71	6.74
Assuming homogeneity (all families)	6.29	6.78	7.29	4.98	7.07	7.52*	7.02	6.28

* Maximum lod score location for bipolar disorders locus.

† The relationship between map distance and θ is nearly linear up to $\theta = 0.15$, so from a practical standpoint, recombination frequencies can be converted directly into map distances with little loss of precision.

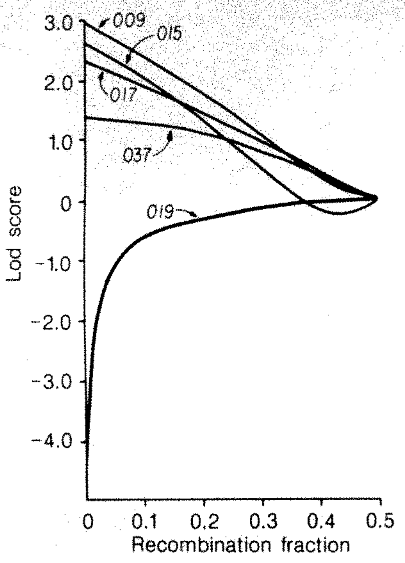

Fig. 4 Lod scores for each pedigree as a function of recombination fraction. Numbers identify the pedigree for each curve.

for linkage between bipolar illness and G6PD deficiency[8] as well as two Sardinian pedigrees[9].

It should be stressed that the pedigrees included in this study represent a select sample, as the study design called for the exclusion of families with male-to-male transmission. In an earlier study[12], we found significant evidence for linkage heterogeneity among previously published pedigrees, and estimated the proportion of X-linked families to be 64%. However, as pedigrees with male-to-male transmission were excluded from the analysis, that figure is not representative of the population as a whole. In a subsequent reanalysis of transmission patterns from family studies we estimated that in general perhaps one-third of the bipolar population carry the X-linked gene[2].

With the advent of molecular genetic techniques, many more polymorphisms have been revealed on the long arm of the X chromosome[30]. These DNA markers, unlike the classical polymorphisms, should render most pedigrees informative for the analysis of X-linkage[30]. This, in turn, should facilitate replication of the X-linkage findings in different populations and resolution of the question of genetic heterogeneity in the bipolar population.

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HPRT-deficient (Lesch–Nyhan) mouse embryos derived from germline colonization by cultured cells

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Embryonal stem (ES) cell lines, established in culture from peri-implantation mouse blastocysts[1–3], can colonize both the somatic and germ-cell lineages of chimaeric mice following injection into host blastocysts[4,5]. Recently, ES cells with multiple integrations of retroviral sequences have been used to introduce these sequences into the germ-line of chimaeric mice[5], demonstrating an alternative to the microinjection of fertilized eggs[6] for the production of transgenic mice. However, the properties of ES cells raise a unique possibility: that of using the techniques of somatic cell genetics to select cells with genetic modifications such as recessive mutations, and of introducing these mutations into the mouse germ line. Here we report the realization of this possibility by the selection *in vitro* of variant ES cells deficient in hypoxanthine guanine phosphoribosyl transferase (HPRT; EC 2.4.2.8), their use to produce germline chimaeras resulting in female offspring heterozygous for HPRT-deficiency, and the generation of HPRT-deficient preimplantation embryos from these females. In human males, HPRT deficiency causes Lesch–Nyhan syndrome[7], which is characterized by mental retardation and self-mutilation.

The structural gene for HPRT is X-linked both in mice[8] and in humans[7]. Thus, male cells are hemizygous and HPRT$^-$ variants, readily selected by resistance to the purine analogue 6-thioguanine[9], arise at relatively high frequency. Although HPRT$^-$ embryonal carcinoma (EC) cells colonized the somatic tissues of chimaeric mice, they failed to colonize the germ line[10]. Failure to colonize the germ line is a common limitation with EC cell lines, although the problem with the HPRT$^-$ cells may have been compounded by the use of a chemical mutagen. Also, selection in the presence of feeder cells causes the loss of resistant variants by transfer of 6-thioGMP from wild-type parental cells through the feeder layer[11] and probably exposes surviving HPRT$^-$ cells to sublethal levels of 6-thioGMP which are mutagenic[12]. We therefore chose to work with an embryo-derived ES cell line, to isolate spontaneous HPRT$^-$ variants,

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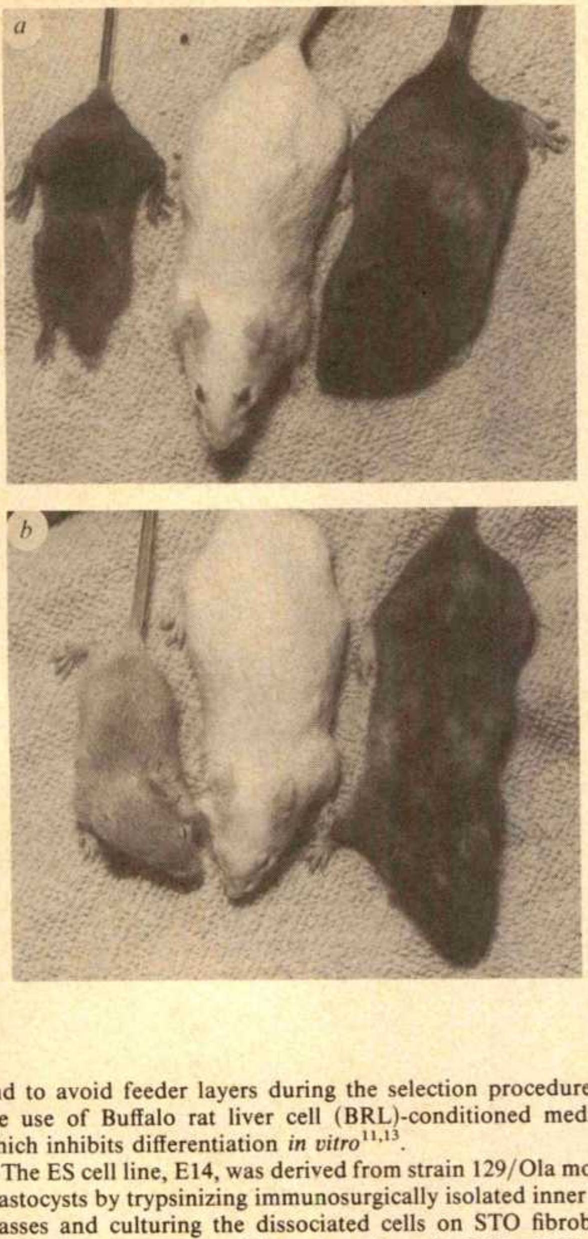

Fig. 1 Male E14TG2a chimaeras and their offspring showing the transmission of coat colour markers. *a*, Male chimaera (right), the BALB/c female to which it was mated (centre) and a host blastocyst-derived offspring (left). *b*, One of the five male chimaeras with complete colonization of the germ line (right), the BALB/c female to which it was mated (centre) and one of the E14TG2a-derived offspring (left). BALB/c mice are homozygous for the albino allele (c/c), so that offspring derived from host sperm are normally pigmented (C/c), but those derived from E14TG2a sperm are pale chinchilla (c^{ch}/c).

Methods. F1 (C57B1/6JLac × CBA/CaLac) female mice (3 to 4 week old) were superovulated by intraperitoneal injection of 7.5 IU pregnant mares serum gonadotrophin at 12:00 followed 48 h later by 5 IU human chorionic gonadotrophin (Intervet), paired with males of the same strain, and examined the following day for copulation plugs (day 1 of pregnancy). Morulae and early blastocysts were flushed from the oviducts and uteri late on day 3 in HEPES-buffered medium M2[23] supplemented with 0.4 mg ml^{-1} bovine serum albumin (BSA) and cultured overnight to the expanded blastocyst stage in medium M16[24] supplemented with 0.4 mg ml^{-1} BSA at 37 °C in an atmosphere of 5% CO_2 in air. Cells were grown on gelatin-coated tissue-culture plasticware (Lux; Flow Laboratories) in BRL-conditioned medium with 20% (v/v) serum (30 ml medium CMβ) incubated for three days with a confluent 175-cm^2 monolayer of BRL cells[25], filtered through a membrane of pore size 0.2 μm, and then diluted with 15 ml fresh medium CMβ and an additional 5 ml fetal calf serum (FCS) at 37 °C in an atmosphere of 95% air, 5% CO_2. Medium CMβ is Glasgow-modified Eagle's medium with 1 × non-essential amino acids and 1 mM sodium pyruvate (Flow Laboratories), 10% (v/v) FCS (Northumbria Biologicals, Cramlington, Northumberland) and 0.1 mM β-mercaptoethanol. Cells were subcultured twice weekly with 0.25% (w/v) trypsin, 1 mM Na_2EDTA, 1% (v/v) chicken serum and monitored periodically for mycoplasma contamination by the method of Chen[26]. No such contamination was detected during this study. Colonies of cells were selected with a micropipette, washed twice in Hanks Balanced Salts solution without calcium and magnesium ions (Imperial Labs), incubated in 0.125% (w/v) trypsin, and finally washed and disaggregated in medium CMβ with 20% (v/v) FCS. Cells were injected into blastocysts using a De Fonbrune micromanipulator and Agla microsyringe. Injected blastocysts were allowed to re-expand in culture for 1 to 2 h before transfer to the uteri of day-3 pseudopregnant recipients.

and to avoid feeder layers during the selection procedure by the use of Buffalo rat liver cell (BRL)-conditioned medium which inhibits differentiation *in vitro*[11,13].

The ES cell line, E14, was derived from strain 129/Ola mouse blastocysts by trypsinizing immunosurgically isolated inner cell masses and culturing the dissociated cells on STO fibroblast feeder cells in BRL-conditioned medium (A.H.H., K.H., M. Jones and M.L.H., manuscript in preparation). Following expansion into mass culture, E14 cells were maintained in BRL-conditioned medium alone. For the isolation of HPRT$^-$ variants, 4.4×10^6 E14 cells were seeded at 7×10^3 cells cm^{-2} in BRL-conditioned medium with 20% serum (see Fig. 1 legend) on gelatin-coated dishes. The cells attached overnight and then the medium was replaced with selective medium containing 10 μg ml^{-1} 6-thioguanine which was changed every 2–3 days. After 13 days, seven surviving colonies were visible. By picking and subculturing in non-selective medium, sublines of undifferentiated cells were established from four of these colonies. These were screened for thioguanine resistance, sensitivity to HAT (hypoxanthine, aminopterin and thymidine)[14], karyotype and ability to differentiate *in vitro*, and the subline E14TG2a was chosen for further study. Like E14 cells, E14TG2a cells had a modal chromosome number of 40 (16 of 20 spreads had the modal number). They were identified as male by the presence of a small uniformly fluorescent chromosome, identified as the Y chromosome[15], after staining with *bis-N, N*″ (6-chloro-2-methoxy-acridin-9-yl)spermidine ($(CMA)_2S$) (which gives similar staining to the spermine derivative[16]). Further, E14TG2a cells differentiated extensively *in vitro* following formation of suspension aggregates in non-conditioned medium and subsequent outgrowth (ref. 13; and A.H.H. *et al.*, in preparation). However, E14TG2a cells were resistant to 10 μg ml^{-1} 6-thioguanine at cell densities at which E14 cells were killed by less than 0.1 μg ml^{-1} of this analogue, and unlike E14 cells they failed to grow in HAT medium. This suggested a deficiency in HPRT, and indeed when this enzyme was assayed directly by the method of Gillin *et al.*[17] no activity was detectable (specific activities: E14, 0.57 ± 0.06 (mean ± s.e.m.) pmol inosinic acid (IMP) per min per μg cell protein; E14TG2a < 0.002 pmol IMP per min per μg cell protein).

Between 5 and 20 HPRT$^-$ E14TG2a cells were injected into each of a series of host F2 (C57B1/6JLac × CBA/CaLac) blastocysts. Of 396 injected blastocysts transferred to recipients which became pregnant, 266 mice (67%) were born. Chimaeras were identified by examining the coat colour of litters at 7–10 days of age. F2 host blastocysts are homozygous for the wild-type allele at the *c* locus (C/C), but 129/Ola-derived E14TG2a ES cells are homozygous for the chinchilla allele (c^{ch}/c^{ch}) which produces a lighter coat colour. If the coat was not chimaeric, blood samples were taken at about 4 weeks for glucose phosphate isomerase isozyme (GPI)[4] analysis (F2 host blastocyst: *Gpi-1*^{b}/*Gpi-1*^{b}; 129/J-derived E14TG2a ES cells: *Gpi-1*^{a}/*Gpi-1*^{a}). However, blood isozyme analysis of 119 of these mice failed to detect any more chimaeras and so this additional screening was discontinued. The incidence of chimaerism ranged from 0 to 38% between experiments, but overall a total of 63 (24%) chimaeras were born. This is comparable with the incidence obtained with other ES lines[4] although lower than recently achieved with one particular line[5].

The distortion of the sex ratio and the pattern of germline

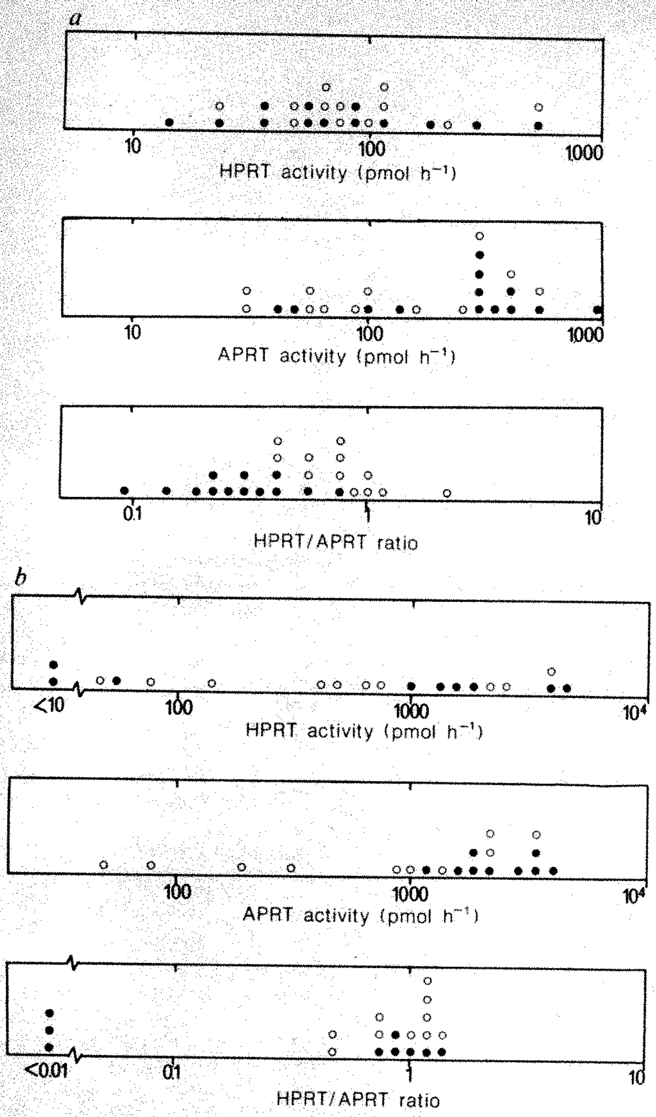

Fig. 2 Distribution of HPRT and APRT activities and their ratios in *a*, single hair follicles and *b*, single intestinal crypts. ●, Presumptive *Hprt*$^+$/*hprt*$^-$ heterozygous females, and ○, controls (male E14TG2a-derived littermate and BALB/c female in (*a*) and BALB/c female in (*b*)). Each data point represents a single follicle or crypt. The vertical co-ordinate of individual data points has no significance other than to avoid superimposition.

Methods. Hairs with intact follicles, and intestinal crypts isolated as described[19] were placed in 10-μl capillary tubes with approximately 5 μl medium M2 supplemented with 0.4 mg ml^{-1} polyvinylpyrrolidone, the column of fluid shaken into the centre and the ends heat-sealed. Samples were freeze-thawed three times in liquid nitrogen and HPRT and APRT activities assayed as described[27].

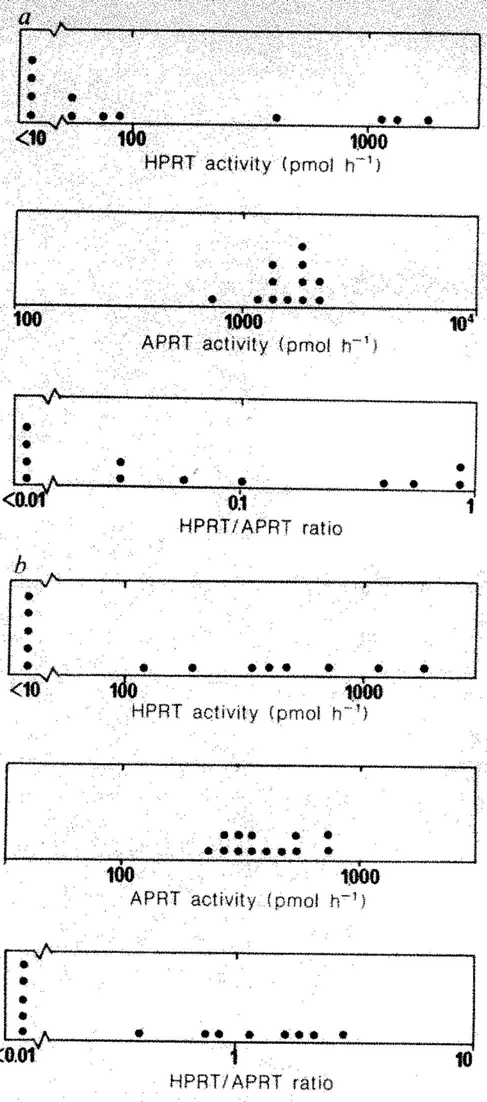

Fig. 3 Distribution of HPRT and APRT activities and their ratios in single day-3 morulae from matings between heterozygous females and wild-type F1 (C57B1/6JLac × CBA/CaLac) males: *a*, litter no. 1; *b*, litter no 2. Each data point represents a single morula. Plotting of points is as in Fig. 2.

transmission is similar to that observed with other male ES cell lines[4,5]. Fifty chimaeras are phenotypically male, and 11 female, by external examination (two mice died before sexing). To date, 19 of 34 male chimaeras test bred with females carrying appropriate markers have shown germline transmission of the c^{ch} marker derived from the HPRT$^-$ E14TG2a ES cells (Fig. 1). With six males the marker is transmitted to all the offspring, and among the other thirteen the proportion of E14TG2a-derived offspring ranges between 1 and 56% and in many cases is higher than previously observed with other lines for partial transmission[4].

On the assumption that the HPRT-deficiency of E14TG2a cells is due to an X-linked structural gene mutation, all female offspring carrying the c^{ch} marker should be heterozygous for HPRT-deficiency and all male offspring wild-type. Human female carriers for Lesch–Nyhan syndrome are identified by determining the ratio of activities of HPRT and the autosomal enzyme, adenine phosphoribosyl transferase (HPRT/APRT ratio), in single hair follicles. Follicles from a single heterozygote are heterogeneous with respect to HPRT/APRT ratio, with normal, completely HPRT-defective and intermediate phenotypes present[18], presumably reflecting the mosaic distribution of HPRT$^-$ cells in the scalp resulting from random X-inactivation. Although we do not see completely HPRT$^-$ follicles in the heterozygous female E14TG2a-derived offspring, presumably because some HPRT$^+$ cells are present in all our samples, the HPRT/APRT ratios show a distribution of values lower than those for wild-type males and females (Fig. 2*a*). Moreover, in a representative heterozygous female, some intestinal crypts, each of which is a clonal population of cells with respect to X-inactivation[19], have undetectable HPRT activity, although the APRT activities fall within the control range (Fig. 2*b*). These results are consistent with the mosaic distribution of HPRT$^-$ cells which would be expected in females generated from spermatozoa carrying an X-linked *hprt*$^-$ mutation, and confirm the germline transmission of the mutant allele. Germline transmission of an *hprt*$^-$ allele derived from an ES cell line subjected to retroviral insertion mutagenesis has been independently achieved by Kuehn *et al.*[20].

Heterozygous females were mated with normal males and their preimplantation embryos assayed at the morula stage. Some of the embryos from each of two such litters showed undetectable levels of HPRT activity (Fig. 3) as would be expected for hemizygous male embryos carrying the mutant allele on their single X chromosome, indicating that the mutant allele can be transmitted through the female germ line and the resulting HPRT-deficient embryos survive to the morula stage. Nine litters from similar matings have been allowed to develop to term. The litter sizes at birth ranged from 8 to 13, with 52 males and 40 females. On the basis of enzyme assay on blood samples from 41 of these mice, 11 out of 21 males and none of 20 females are HPRT-deficient. There is therefore no significant loss of HPRT-deficient male embryos *in utero*. No postnatal deaths have yet occurred in these litters, which are now between three and five weeks old.

In humans, HPRT-deficient males exhibit a characteristic pattern of behavioural abnormalities (Lesch–Nyhan syndrome), of which the principal features are mental retardation, spastic cerebral palsy, choreoathetosis, and self-mutilative biting[7]. There is circumstantial evidence that this is caused by the development of lesions in the dopamine-mediated neural pathways of the basal ganglia of the brain[21], but the absence of any known case of HPRT-deficiency in an experimental animal has so far prevented a definitive test of this hypothesis. This may now be possible with these HPRT-deficient mice. Although no overt behavioural abnormalities have been noted either in HPRT$^-$ progeny of heterozygous females (up to their present age of five weeks) or in male chimaeras with apparently extensive HPRT$^-$ cell contributions, detailed behavioural investigations have yet to be carried out. HPRT-deficient mice may also be valuable for evaluating therapeutic procedures such as chronic L-DOPA therapy or neural transplants[21], and as a model system for rescuing defective embryos by cell replacement or gene therapy[22]. In addition, the use of HPRT-deficient mouse embryos will facilitate the development of techniques for the preimplantation diagnosis of Lesch–Nyhan syndrome and other inherited diseases using biopsies of early human embryos. Finally, the general approach described here for introducing genetic modifications into the mouse germ line be applicable to other genetic lesions of developmental and clinical interest.

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A potential animal model for Lesch–Nyhan syndrome through introduction of HPRT mutations into mice

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The human Lesch–Nyhan syndrome is a rare neurological and behavioural disorder, affecting only males, which is caused by an inherited deficiency in the level of activity of the purine salvage enzyme hypoxanthine-guanosine phosphoribosyl transferase (HPRT)[1–3]. How the resulting alterations in purine metabolism lead to the severe symptoms characteristic of Lesch–Nyhan patients is still not understood[3,4]. No mutations at the *Hprt* locus leading to loss of activity have been described in laboratory animals. To derive an animal model for the Lesch–Nyhan syndrome, we have used cultured mouse embryonic stem cells[5], mutagenized by retroviral insertion and selected for loss of HPRT activity, to construct chimaeric mice[6,7]. Two clonal lines carrying different mutant *Hprt* alleles have given rise to germ cells in chimaeras, allowing the derivation of strains of mutant mice having the same biochemical defect as Lesch–Nyhan patients. Male mice carrying the mutant alleles are viable and analysis of their cells shows a total lack of HPRT activity.

To increase the frequency of mutations in the X-linked *Hprt* gene in a male embryonic stem cell line, multiple infection with a retroviral vector was carried out[7,8]. HPRT$^-$ variants were isolated from infected stem cell populations using 6-thioguanine (6-TG)[9]. From a total of 10^7 originally infected cells, four 6-TG-resistant clones were isolated, TG^{R}4, 5, 6 and 7. In a separate experiment an additional 6-TG-resistant clone, TG^{R}3, was recovered from 5×10^6 originally infected cells. Selections of 10^8 uninfected cells have not yielded any HPRT$^-$ clones. All five of these clones were judged to be HPRT$^-$, in that each of the 6-TG-resistant clonal lines was killed within three days in HAT (hypoxanthine, aminopterin and thymidine) medium[10] but showed equivalent plating efficiencies in 6-TG medium and normal medium.

Southern analysis of DNA prepared from each of the mutant clones revealed alterations in the restriction fragment pattern of the *Hprt* gene[11], as expected if gene disruption by retroviral integration had occurred. Using enzymes that cut within the locus, an identical altered pattern was seen for the *Hprt* gene in clones TG^{R}4, 5, 6 and 7. This alteration is in the 3′ region of the gene between exons 5 and 6 (Fig. 1). Reprobing with the *neo* gene to determine the integration pattern of the viral vector has shown that these four clones are derived from one mutated cell, the daughter cells of which had been infected several more times before 6-TG selection. Southern analysis of the *Hprt* gene in TG^{R}3 has revealed an alteration in the 5′ region of the gene within the *Hprt* locus but does cut the vector. *Kpn*I digestion of TG^{R}3, 4 or 6 DNA produces two novel fragments rather than

within the *Hprt* locus but does cut the vector. *Kpn*I digestion of TG^{R}3, 4 or 6 DNA produces two novel fragments rather than

the one fragment of 50 kilobases (kb) seen with wild-type DNA (Fig. 1*D*). The appearance of two novel fragments implies that a virus has integrated within the gene thereby introducing *Kpn*I sites into the locus (Fig. 1*E*). This analysis supports the conclusion that these clones are HPRT$^-$ as a result of insertional mutation.

Cells from three of the HPRT$^-$ clonal lines, TG^{R}3, 4 and 6, were used to produce chimaeric animals (see Table 1). The contribution of the injected stem cells to somatic tissues of the chimaeras was extensive and uniformly high. The expected

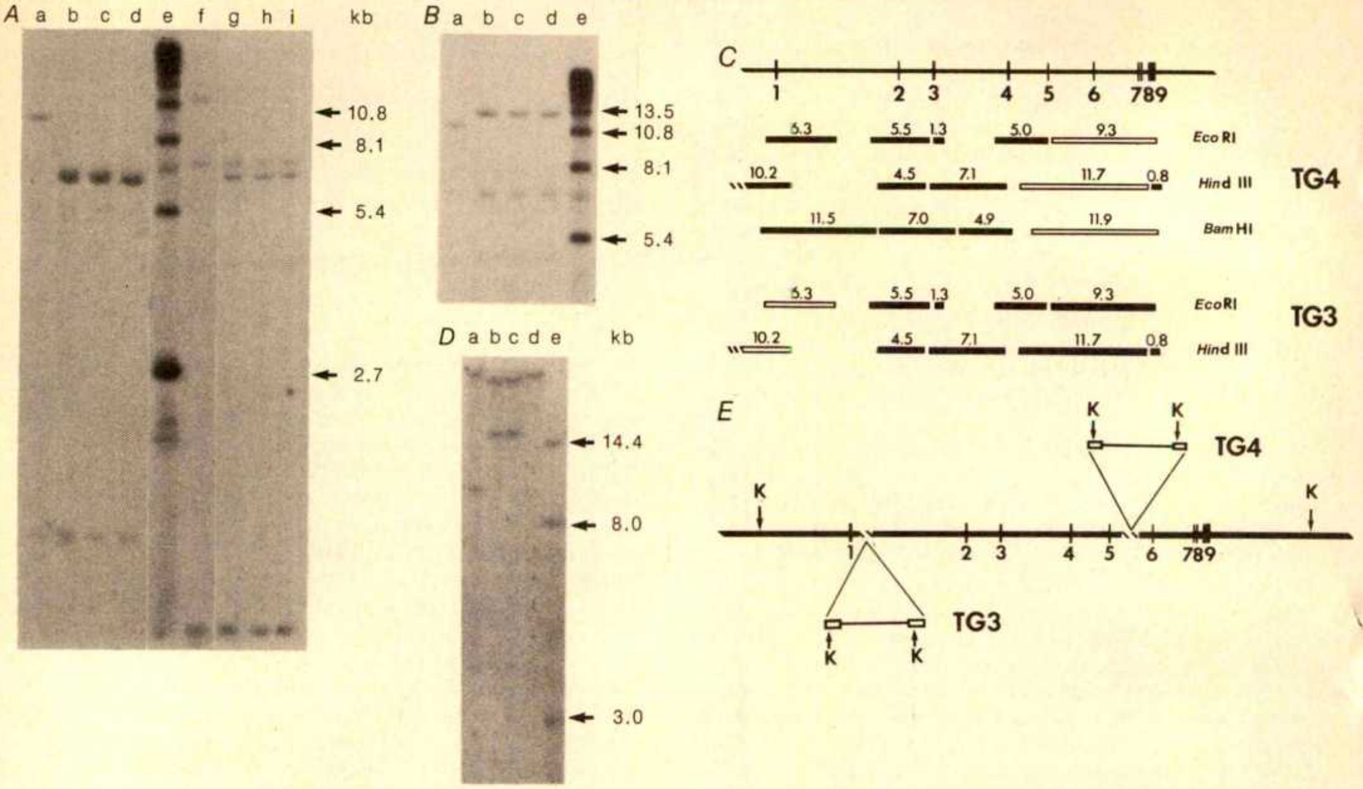

Fig. 1 Southern analysis of 6-TG-resistant clonal lines. DNA samples (~3 μg), prepared from stem cells passaged twice on gelled plates to reduce feeder-cell numbers, were restriction digested, fractionated on agarose gels, transferred to nylon membranes, hybridized with nick-translated pHPT5[27], washed and autoradiographed using standard procedures. *A*, *Eco*RI and *Hin*dIII analysis is shown in lanes a–d and f–i respectively. Lanes a and f, wild-type DNA: lanes b and g, clone TG^{R}4; lane c, clone TG^{R}5; lanes d and h, clone TG^{R}6; lane i, clone TG^{R}7. The sizes of the bands of the size marker lane(e) are shown at the side in kb. *B*, *Bam*HI analysis. Lane a, wild-type DNA; lane b, TG^{R}4; lane c, TG^{R}5; lane d, TG^{R}7; lane e, size marker, sizes are shown in kb beside the lanes. *C*, map of the wild-type mouse *Hprt* gene[11]. Exons are numbered sequentially from the 5′ end of the gene. Fragments produced by the restriction enzymes used in this Southern analysis and that hybridize to the full-length HPRT cDNA probe are shown below the map as dark boxes. The signal strength of some fragments, such as the 6.3-kb *Eco*RI fragment, is low in the blot shown above owing to the small size of the exon contained within the fragment. The open boxes refer to wild-type restriction fragments which are missing in DNA from clones TG^{R}4, 5, 6 and 7 (labelled TG4). The fragments missing in DNA from clone TG^{R}3 are shown as open boxes below the map for TG^{R}4, 5, 6 and 7. *D*, *Kpn*I analysis of DNA from the TG^{R}3, TG^{R}4 and TG^{R}6 clonal lines to determine retroviral integration into the *Hprt* gene. Lane a, TG^{R}3 DNA; lane b, TG^{R}4 DNA; lane c, TG^{R}6 DNA: lane d, wild-type DNA; lane e, *Pst*I digest of wild-type DNA to provide size markers (sizes shown at the side). *E*, retroviral integration into the *Hprt* locus introduces novel *Kpn*I sites (designated K) into the gene, producing two *Kpn*I restriction fragments which hybridize to the cDNA probe in a Southern analysis. As shown in *D*, wild-type DNA has only one 50-kb *Kpn*I fragment whereas TG^{R}3, 4 and 6 have two *Kpn*I fragments, the expected result of viral integration.

distortion in the overall sex ratio was observed in the TG^{R}3 and TG^{R}6 series of chimaeras[6], but was lacking in the population of chimaeras produced using TG^{R}4 cells. Karyotype analysis of the TG^{R}4 clonal line showed that 20 out of 41 mitoses scored by G-banding[12] lacked a Y chromosome, whereas TG^{R}3 and TG^{R}6 were normal. As XO cells in a female mouse can give rise to gametes[13], the female chimaeras of the TG^{R}4 series as well as all the male chimaeras were test bred. TG^{R}4-derived cells have colonized the germ line of one female and seven male chimaeras. TG^{R}3-derived cells have contributed to the germ line of three male chimaeras (see Table 1).

Southern analysis of DNA prepared from five of the female progeny of the female germline chimaera, TG.4.52, has shown that two of these F$_1$ animals have a restriction fragment pattern diagnostic for both the altered allele and the normal *Hprt* gene. The other three F$_1$ females have only the normal fragment pattern (Fig. 2). Karyotype analysis of primary fibroblast cultures derived from the F$_1$ females has shown that the animals with only the normal *Hprt* pattern have a 39, XO chromosomal constitution. In addition to showing that the altered allele has been established in the mouse germ line, this analysis demonstrates that the germ cells of the TG.4.52 chimaera are derived from XO TG^{R}4 cells. Southern analysis of female F$_1$ animals sired by the germline chimaeras of the TG^{R}3 series has demonstrated that this allele has also been established in the germ line (data not shown). This allele has been named *Hprt-b*^{m1} and the allele carried by the TG^{R}4-derived animals has been named *Hprt-b*^{m2}.

To determine whether these two newly established *Hprt* alleles continue to cause a mutant phenotype the primary fibroblast cultures derived from the heterozygous F$_1$ females were further analysed. Owing to random X-chromosome inactivation a percentage of these heterozygous cells should lack HPRT activity[14,15]. After two weeks in 6-TG medium, heterozygous cells from animals bearing either allele were not impaired in growth. Control fibroblasts derived from albino littermates and from the XO F$_1$ animals carrying only the normal X-chromosome were killed within one week. As a further assay of HPRT activity, unselected primary fibroblasts were plated at low density in medium containing ^{3}H-hypoxanthine[14,15]. Incorporation of the labelled compound into nucleic acids is dependent on HPRT activity. Autoradiography has shown that whereas 100% of the HPRT$^+$ control cells were found to be labelled, approximately 40% of the cells derived from the heterozygous F$_1$ animals were unlabelled (Fig. 3). This and the first test confirm that cells expressing these altered *Hprt* alleles are deficient in HPRT activity.

To derive hemizygous mutant males, the F$_1$ females heterozy-

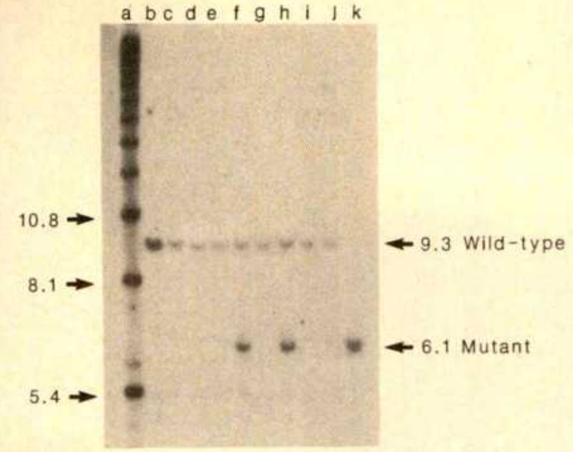

Fig. 2 Southern analysis of DNA from F_1 animals to confirm genetic transmission of the *Hprt-b*^{m2} allele. DNAs were prepared from tail biopsies taken at approximately two weeks of age, by a modification of the method of Hanahan (from ref. 28). Samples (~2 μg) were digested with *Eco*RI and fractionated on a 0.8% agarose gel. Transfer and subsequent steps were as in Fig. 1. The sizes of fragments in the marker lane a are shown beside the lanes. Lane b, wild-type DNA from a female animal; lanes c–e, DNA from male F_1 animals produced by TG3.15, TG4.5 and TG4.13 respectively; lanes f–j, DNA from F_1 females produced by TG4.52, the female germline chimaera; lane k, DNA from the TG^{R}4 clonal line. Two of the F_1 females have the *Eco*RI fragment of ~6.1 kb diagnostic for the *Hprt-b*^{m2} allele, whereas the other three have only the normal pattern and from signal intensity appear to have only one copy of the *Hprt* gene.

gous for the mutant *Hprt* alleles (carrier females) have been bred and approximately equal numbers of male and female F_2 animals obtained (77 females and 66 males). Prenatal lethality due to HPRT deficiency would have led to a 50% reduction in the number of male offspring. The TG.4.52 chimaera has an XO germ line, with the X chromosome carrying the *Hprt-b*^{m2} allele. This female germline chimaera has produced male progeny which are obligate hemizygous mutants. This provides a further demonstration of the pre- and post-natal viability of mice hemizygous for these mutant *Hprt* alleles.

Following growth in ^{3}H-hypoxanthine, an autoradiographic analysis of primary fibroblast cultures derived from the hemizygous mutant males produced by TG.4.52 has shown all cells to be completely unlabelled, indicating a total deficiency of HPRT activity (Fig. 3). At eleven weeks of age these males are apparently healthy and remain free from any gross neurological symptoms.

Using HPRT$^-$ mutant stem cells selected in tissue culture we have succeeded in deriving strains of mice carrying mutant *Hprt* alleles in all their cells. Construction of germline chimaeras with stem cells carrying a spontaneous mutation of the *Hprt* gene has been independently achieved by Hooper *et al.*[16]. An earlier attempt to derive HPRT$^-$ mice using HPRT$^-$ embryonal carcinoma (EC) cells was not successful, as this clonal line was not capable of colonizing the germ line of chimaeric mice[17].

In human patients with Lesch–Nyhan syndrome there is variation in the level of residual HPRT activity, with the severity of the symptoms usually inversely related to these levels[3]. One of the most sensitive ways of detecting the low residual levels is through autoradiographic analysis of primary fibroblast cultures[14,15,18]. Enzyme assays done directly on cell lysates can give completely negative results, but fibroblasts from the same patient will often show labelling[19]. To assay HPRT function in animals carrying these newly established alleles, we have applied this test to primary fibroblasts derived from heterozygous female mice and have demonstrated for both alleles that the mutant phenotype originally selected in culture continues to be exhibited in tissues of the mouse. The complete lack of labelling

Table 1 Characteristics of mouse chimaeras

Chimaeras derived from HPRT$^-$ clonal lines

Clonal line	Injected blastocysts	Animals born	Chimaeras Total	Male	Female
TG^{R}3	266	157	66	50	16
TG^{R}4	228	143	94	52	42
TG^{R}6	135	84	43	31	12

Test breeding of chimaeras from HPRT$^-$ clonal lines

Clonal line	Males test bred	Females test bred	Fertile males	Fertile females	Germline chimaeras
TG^{R}3	44	—	36	—	3 males
TG^{R}4	36	25	27	19	8 {7 males 1 female
TG^{R}6	15	—	6	—	—

Breeding data of germ-line chimaeras

Chimaera	No. of litters	Albino	Pigmented Total	Male	Female
Male TG3.15	2	24	1	1	0
Male TG3.1	6	46	1	0	1
Male TG3.22	6	58	2	1	1
Male TG4.5	3	37	2	1	1
Male TG4.11	2	18	1	0	1
Male TG4.13	3	30	1	1	0
Male TG4.14	2	22	1	1	0
Male TG4.24	1	0	6	4	2
Male TG4.53	2	21	1	1	0
Male TG4.57	1	12	1	1	0
Female TG4.52	3	0	8	2*	6
Total	31	268	25	13	12†

* These two male F1 animals are hemizygous for *Hprt* mutant alleles.

† Female F_1 animals carry *Hprt* mutant alleles.

Three HPRT$^-$ clonal lines were used to produce chimaeras. The chimaeric populations obtained from the TG^{R}3 and TG^{R}6 lines show a sex-ratio distortion effect. The sex ratio of the chimaeras obtained from the TG^{R}4 line was not distorted. Sexually mature chimaeric animals were caged with albino MFI mates and the resulting litters were scored for pigmented progeny. GPI (glucose phosphate isomerase) analysis of blood samples provided confirmatory evidence of the transmission of the EK.CCE-derived *Gpi-1*^{c} allele. As the TG^{R}4 clonal line is composed of both XO and XY cells both male and female chimaeras were test bred. In the other series only males were set up for test breeding. In all three TG^{R}3 germline chimaeras (bottom part of table) only a minor proportion of functional sperm is derived from the cultured cells, as reflected in the small number of pigmented progeny obtained. The male germline chimaeras from the TG^{R}4 line fall into two categories. Six animals show a partial transmission of the cultured cell genotype, but all offspring from one animal have inherited the dominant coat colour marker. The single female TG^{R}4 germline chimaera has produced a total of 8 offspring (6 females, 2 males) all of which carry the dominant coat-colour phenotype. F_1 progeny were confirmed as carrying the mutant alleles by autoradiographic analysis of cultured fibroblasts and by Southern analysis of DNA obtained from tail biopsies.

Methods. The EK.CCE cell line was derived from a single XY blastocyst-stage embryo of the inbred 129/Sv//Ev strain, homozygous for the *C* (pigmented) and the *Gpi-1*^{c} alleles, and maintained on STO feeder layers. Before infection the cell line was tested for its ability to form chimaeras by introducing 10–12 individual cells into blastocyst stage embryos as described[6]. Host blastocysts were obtained from MFI outbred strain mice, homozygous for the *c* (albino) and for the *Gpi-1*^{b} alleles. Live-born animals were sexed at birth and scored later as chimaeric by the presence of coat and eye pigmentation and by GPI analysis. This line has demonstrated 100% chimaera formation and functional germline colonization in 38% of the fertile males. Retroviral infection was designed to produce more than 10 copies per cellular genome in the minimum time. Psi2 cells[25] producing the MPSV mos^{-1} neo vector[26] at a titre of 10^5 ned^R units per ml per 12 h on 3T3 cells were mitotically inactivated by mitomycin treatment and seeded at a density of 10^6 cells on a 6-cm dish. The following day stem cells were plated at a density of 10^6 cells per dish on 10 dishes and cultured on these feeder layers for two days (~2–3 cell doublings). The infected stem cells were collected by trypsinization, mixed and expanded on STO feeder plates. HPRT$^-$ variants were selected by co-plating single-cell suspensions of infected stem cells and mitotically inactivated HPRT$^-$ STO cells directly into medium containing 10^{-6} M 6-TG at a ratio of 2×10^6 stem cells to 5×10^6 STO cells per 10-cm dish on 50 dishes. Colonies growing after two weeks in 6-TG were returned to normal medium, colony-cloned and expanded into clonal cell lines. Chimaeras were made from the TG^{R}3, 4 and 6 HPRT$^-$ clonal lines by blastocyst injection.

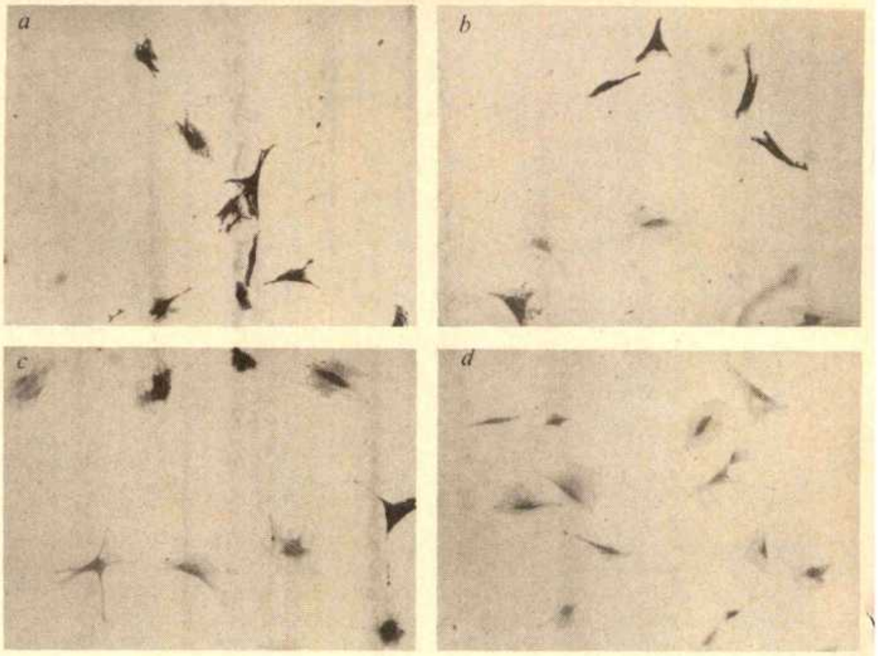

Fig. 3 Autoradiography of cultured primary fibroblasts following growth in medium containing ^{3}H-hypoxanthine. *a*, Cells derived from XO F_1 female, produced by TG4.52; *b*, cells derived from F_1 female produced by TG3.1; *c*, cells derived from XX F_1 female produced by TG4.52; *d*, cells derived from F_1 male produced by TG4.52.

Methods. Tail biopsies were taken aseptically at birth, minced finely and placed in a drop of medium supplemented with 20% newborn calf serum and non-essential amino acids in a 3-cm dish. A cover-slip was put over the drop and the dish was incubated for several days before collecting fibroblast outgrowths by trypsinization. For the uptake experiment, 10^4 cells were plated on 7-cm Petri-perm culture dishes in 4 ml supplemented medium containing 10 μCi ml^{-1} ^{3}H-hypoxanthine (8.2 Ci mmol^{-1}). After 24 h, plates were washed twice in phosphate-buffered saline (PBS), fixed in 3 : 1 methanol/acetic acid vapour, extracted twice with ice-cold 10% trichloroacetic acid (TCA) and washed overnight in running water. Plates were dipped in Kodak NTB-2 emulsion and exposed for 4 days.

of the hemizygous mutant mouse cells in our autoradiographic analysis suggests that there is not even residual HPRT activity in these animals. The DNA polymorphisms associated with each allele provide a convenient molecular marker for identifying animals carrying these mutant *Hprt* genes, obviating the need for the derivation and testing of primary fibroblast cultures.

As is the case in humans, the lack of HPRT activity does not lead to prenatal mortality in mice. The postnatal development and maturation of the male F_1 and F_2 animals carrying the mutant alleles are being followed to determine whether the metabolic, neurological and behavioural symptoms characteristic of the Lesch–Nyhan syndrome are in any way reproduced in an HPRT$^-$ mouse, thereby allowing these animals to serve as models for the human disease. In the human, Lesch–Nyhan males often do not display any behavioural or neurological symptoms until many months after birth[3]; therefore it may be some time before these mice display any gross abnormalities. Many promising steps towards gene therapy for the Lesch–Nyhan syndrome have been made[20-22], and HPRT$^-$ mice may provide a useful whole-animal system for advancing this work.

The success of this approach for the genetic manipulation of the mouse opens up the possibility of deriving strains carrying specifically induced alterations in other genes, as long as the mutant cells can be selected from the population of infected cells or easily screened for. It may also eventually be possible to produce specific alterations in endogenous genes through homologous recombination with cloned copies modified *in vitro*[23,24].

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Alternative pathway activation of T cells by binding of CD2 to its cell-surface ligand

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Activation of resting T lymphocytes is initiated by the interaction of cell-surface receptors with their corresponding ligands. In addition to activation through the CD3 (T3)-Ti antigen–receptor complex[1], recent experiments have demonstrated induction of T-cell proliferation through the CD2 (T11) molecule[2–4], traditionally known as the erythrocyte(E)-receptor, through which T cells can bind red blood cells (RBC)[5–7]. This 'alternative pathway' of T-cell

activation[2] was observed *in vitro* in response to combinations of anti-CD2 monoclonal antibodies (mAbs) that bind to distinct epitopes of CD2, such as mAbs against $T11_2$ plus $T11_3$ (ref. 2). The physiological importance of this activation pathway can be assessed only by studying the effects of a naturally occurring ligand of CD2 on T-cell activation. We have recently described such a ligand, a glycoprotein of apparent relative molecular mass 42,000 (M_r 42K) that is expressed on all blood cells and some other tissues[9,11]. Here we demonstrate that binding of this cell surface molecule, termed T11 target structure or T11TS, to CD2 (T11) induces reactivity in resting T cells to a mitogenic stimulus given by a mAb to the $T11_3$ determinant or by submitogenic concentrations of anti-$T11_{2+3}$ mAbs. Thus, one of the signals required for T-cell activation through the alternative pathway is provided by the interaction of CD2 with a naturally occurring complementary cell-surface molecule.

Our experimental approach was based on the hypothesis that CD2 is a cell-interaction molecule with a complementary ligand, T11TS, that is expressed not only on RBC, where it is involved in E-rosetting[8], but also on other cell types, where it plays a role in T-cell activation[9]. CD2 binds to both human and sheep RBC[8,10], so it apparently recognizes its ligand across this species barrier. We have identified sheep T11TS with a mAb to sheep RBC (SRBC) that completely blocks their binding to human or sheep T cells[8]. This mAb, called L180/1, detects T11TS on a variety of cell types, including most if not all blood cells[9,11]. Its functional involvement in T-cell activation was suggested by its ability to block the sheep mixed lymphocyte reaction[9]. Although mAb L180/1 recognizes a sheep-specific determinant on T11TS[8], recent evidence has indicated that the lymphocyte function associated antigen (LFA)-3[12] may represent the human form of T11TS. Thus, anti-LFA-3 mAb blocks binding of human RBC to T cells[21], and the LFA-3 antigen has a cellular distribution similar to T11TS[12]. Moreover, a polyclonal rabbit anti-sheep T11TS antiserum inhibits human autologous E-rosetting (G.T. and T.H., in preparation). Evidence that T11TS and LFA-3 share antigenic determinants is shown in Fig. 1. Human RBC were stained with a mAb to LFA-3 (from Dr T. Springer) in the presence or absence of rabbit anti-T11TS antiserum or preimmune serum. Flow cytometric analysis revealed that the anti-T11TS antiserum, but not the preimmune serum, completely blocks the binding of the anti-LFA-3 mAb to human RBC, indicating that it contains antibodies that mask the LFA-3 determinant. Reactivity of mAbs to human RBC unrelated to the LFA-3 molecule was not affected by the antiserum (not shown). Taken together with the functional data mentioned above, this is a strong indication that T11TS and LFA-3 are both functional and structural homologues in sheep and man, respectively.

The presence of T11TS/LFA-3 on all types of blood cells, including the responding T cells, makes it difficult to pinpoint the site of action of anti-T11TS or anti-LFA-3 antibodies in T-cell activation studies. This problem can be overcome, however, if human T cells are used as responder cells and SRBC as a source of T11TS-expressing cells together with a sheep-specific anti-T11TS mAb. Accordingly, we have employed SRBC, mAb L180/1 and the anti-$T11_2$ and anti-$T11_3$ mAbs to investigate whether binding of a naturally occurring ligand to CD2 provides an activation signal to human T lymphocytes. Nylon-wool enriched T lymphocytes from peripheral blood were cultured with or without SRBC in the presence of either a combination of anti-$T11_2$ and anti-$T11_3$ mAbs, or anti-$T11_3$ alone to see if SRBC could replace the requirement for the $T11_2$-specific mAb (Fig. 2). This approach was chosen because anti-$T11_2$ or anti-$T11_3$ antibodies by themselves are not mitogenic, but together activate T lymphocytes polyclonally[2]. If binding of T11TS to CD2 is one of the steps physiologically involved in this 'alternative pathway', such a signal may be expected to replace the requirement for one of the two anti-T11 mAbs in T-cell activation through the CD2. As expected, a proliferative response to anti-$T11_2$ plus anti-$T11_3$, but not to either antibody by itself, was observed in the absence of SRBC (Fig. 2, experiment 1). In contrast, anti-$T11_3$ alone could induce proliferation if SRBC were present. Addition of either intact mAb L180/1 or of its Fab fragment completely abolished the stimulatory effect of SRBC, indicating that binding of T11TS to CD2 was essential. In addition, a vigorous proliferative response to a submitogenic concentration of $T11_2$ plus $T11_3$-specific mAbs was observed in the presence of SRBC (Fig. 2, experiment 2). Again, this response was abolished by mAb L180/1. It is unlikely that this inhibition is due to a non-specific effect exerted by, for example, antibody-coated SRBC because a different SRBC-specific mAb of the same isotype and used at the same titre as mAb L180/1 was unable to reverse the co-stimulation by SRBC (Fig. 3). Thus, mAb L180/1 appears to neutralize the stimulatory effect of T11TS. Figure 3 also demonstrates that T11TS-dependent induction of the alternative pathway is a dose-dependent phenomenon: the response to SRBC plus anti-$T11_3$ increases steeply with the addition of greater numbers of SRBC. Again, this effect is completely blocked by the addition of anti-T11TS mAb.

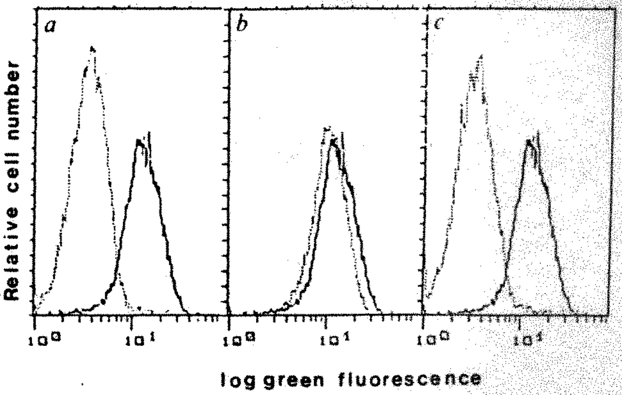

Fig. 1 Rabbit anti-T11TS antiserum blocks binding of anti-LFA-3 mAb to human RBC. *a*, No rabbit serum. Straight line, anti-LFA-3; dotted line, negative control. *b*, Anti-LFA-3 in the absence (straight line) or presence (dotted line) of preimmune serum. *c*, Anti-LFA-3 in the absence (straight line) or presence (dotted line) of rabbit anti-T11TS serum.

Methods. Human RBC (5×10^5) were treated with the mouse mAb TS2/9[12] against LFA-3 at 1 μg ml^{-1} or with a negative control mAb of the same isotype in the presence or absence of rabbit preimmune serum or anti-T11TS immune serum (1:10 final dilution). After 30 min on ice, the cells were washed, stained for 30 min with fluorescein isothiocyanate (FITC)-conjugated Fab_2 rabbit anti-mouse immunoglobulin (DAKO, 1:50), and analysed on a FACScan flow cytometer (Becton-Dickinson) using a light scatter gate for individual RBC and logarithmic amplification for fluorescence. Rabbit anti-T11TS antiserum was prepared by injecting 50 μg aluminium hydroxide-adsorbed sheep T11TS purified to homogeneity by immune-affinity and preparative SDS-polyacrylamide gel electrophoresis[11], followed by five injections of 50 μg T11TS in soluble form at 3-week intervals.

These results show that the interaction of CD2 with its naturally occurring complementary cell-surface molecule provides an activation signal to T lymphocytes through the 'alternative pathway'. At first sight, it appears paradoxical that despite the expression of T11TS (or LFA-3) on leukocytes, including T cells, SRBC are required to demonstrate the functional effects of CD2–T11TS interaction. The inability of T-lymphocyte-borne T11TS to complement anti-$T11_3$ or submitogenic concentrations of anti-$T11_{2+3}$ in T-cell activation is most probably due to the high negative net charge of resting peripheral T cells[13] that prevents physical contact between them[14]. This interpretation is supported by the fact that despite the expression of LFA-3 on human RBC, the formation of autologous E-rosettes does not occur *in vivo* but requires reduction of negative surface charge of either the lymphocytes or the RBC[15] by neuraminidase

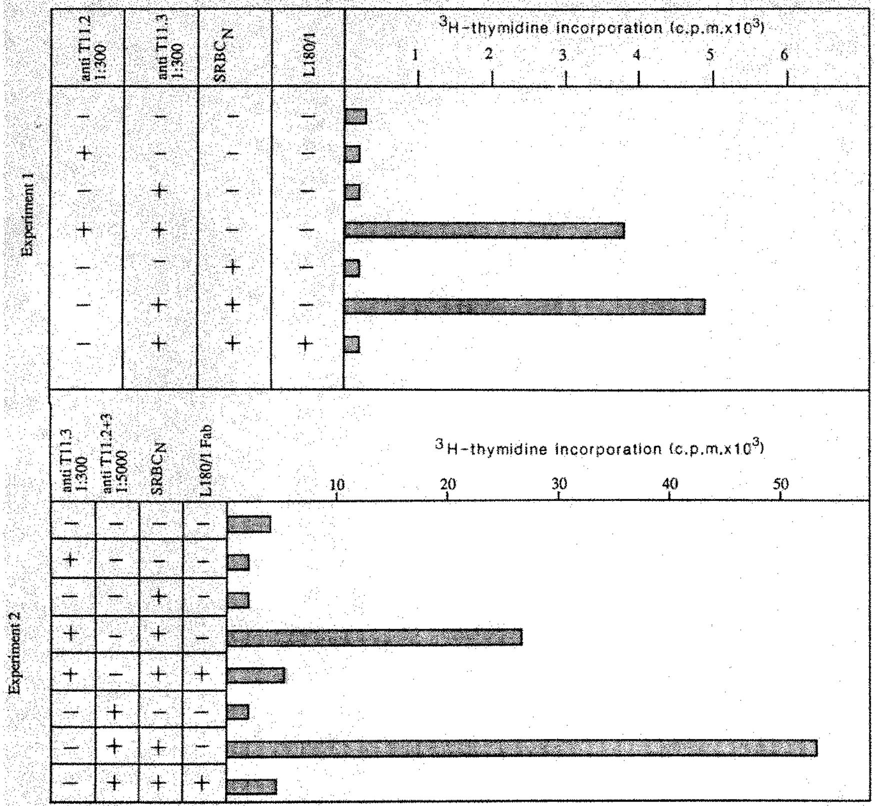

Fig. 2 Co-stimulatory effect of SRBC is inhibited by anti-T11TS mAb. Peripheral blood mononuclear cells (PBMNC) were isolated by Ficoll density centrifugation and passed over nylon wool to enrich for T lymphocytes. Cells (10^5) were cultured for 4 days in round-bottom microwells in a final vol. of 0.2 ml RPMI 1640 medium containing 10% heat-inactivated human serum, antibiotics and 2% glutamine, with the additions indicated. Ascites fluids containing anti-T11$_2$ and anti-T11$_3$ mAbs were used at the concentrations given. L180/1 was purified antibody used at 10 μg ml^{-1} Antigen-binding fragment (Fab) of L180/1 (10 μg ml^{-1}) was prepared by papain digestion and shown to contain no intact antibody. SRBC (10^6 per well) were treated with neuraminidase (to give SRBC$_N$) to reduce surface charge and promote E-rosetting. At the onset of culture, the plates were centrifuged for 5 min at 200*g* to facilitate binding of SRBC to T lymphocytes. Cultures were pulsed with 1 μCi ^{3}H-thymidine for the last 16 h of culture. Standard deviations of results from triplicate cultures were below 15%.

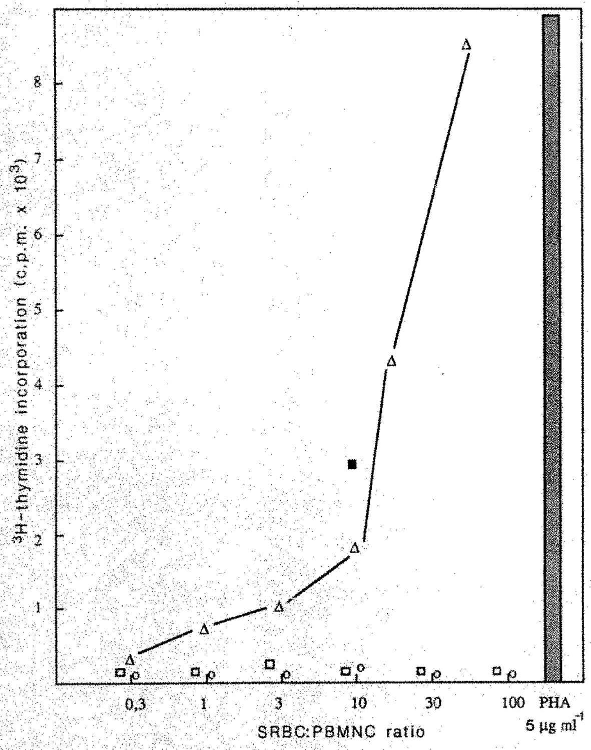

Fig. 3 Dose-response relationship of SRBC-induced T-cell activation by the alternative pathway. Responses are shown to: □, SRBC alone; Δ, SRBC + anti-T11$_3$; ○, SRBC + anti-T11$_3$ + L180/1; ■, SRBC + anti-T11$_3$ + 1/2. Culture conditions were as in Fig. 2 except that SRBC$_N$ were added at 3×10^4 to 1×10^7 per well, as indicated. Anti-T11$_3$ was used at 1:300 dilution of ascitic fluid. Like L180/1, mAb 1/2 is an immunoglobulin G (IgG)$_1$ antibody reactive with both SRBC and sheep leukocytes, but is is not specific for T11TS. Also shown is the response to 5 μg ml^{-1} phytohaemagglutin (PHA, Sigma). PBMNC, peripheral blood mononuclear cells.

treatment[16]. SRBC bind readily to resting T cells through CD2–T11TS and thus provide a system to study the functional effects of this interaction at the level of the resting T cells. We suggest that the function performed by SRBC in this experimental situation is performed *in vivo* by other cell types to which T lymphocytes or thymocytes bind through CD2, such as activated antigen-presenting cells or thymic epithelial cells[17].

Despite the clear-cut functional effect of CD2–T11TS interaction, we are far from understanding the whole sequence of events involved in T-cell activation through CD2. In particular, the natural counterpart to the 'second signal' required in addition to T11TS (here given by anti-T11$_3$ or submitogenic concentrations of anti-T11$_{2+3}$) remains unresolved. A requirement for two signals in T-cell activation through CD2 was also apparent in the recent experiments of Holter *et al.*[4], who confirmed that anti-CD2 mAbs induce T-cell proliferation only if added in pairs. Interestingly, they also found that in the presence of the protein kinase C activator phorbol myristate acetate, individual anti-CD2 mAbs can also activate T lymphocytes. The latter finding suggests that under physiological conditions, the interaction of CD2 with T11TS could also be complemented by a CD2-unrelated activation pathway. Sub-optimal signalling through the antigen–receptor complex has recently been shown to synergize with an individual anti-CD2 mAb in T-cell activation[18]. Further support for this view comes from the recent finding that binding of CD2 to the human LFA-3 molecule in cytotoxic T lymphocyte (CTL)-target cell conjugation is also by itself insufficient to trigger cytolysis[19]. One possibility is that an additional signal required for T-cell activation through CD2 is a lymphokine. The results of Larsson *et al.*[20], who before the discovery of the CD2 described acquisition of reactivity to 'mitogenic factors' as a consequence of E-rosetting, are compatible with such a mechanism. In conclusion, the interaction of CD2 with the naturally occurring ligand T11TS provides an activation signal to T lymphocytes which by itself is insufficient to trigger T-cell activation. How this step fits into the series of events that are part of physiologically occurring T-cell responses is currently under investigation.

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Ion channels activated by inositol 1,4,5-trisphosphate in plasma membrane of human T-lymphocytes

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Hydrolysis of membrane-associated phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)-P_2) to water soluble inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3) is a common response by many different kinds of cells to a wide variety of external stimuli (see refs 1 and 2 for review). Ins (1,4,5)P_3 is a putative second messenger which increases intracellular Ca^{2+} by mobilizing internal Ca^{2+} stores, a hypothesis which has been substantiated by studies with chemically permeabilized cells[3–13] and with isolated microsomal membrane fractions[11,12–16]. But the possibility that Ins(1,4,5)P_3 could induce in intact cells an influx of external Ca^{2+} through transmembrane channels, originally hypothesized by Michell in 1975[17], has never been directly tested. We report here single-channel recordings of an Ins(1,4,5)P_3-activated conductance in excised patches of T-lymphocyte plasma membrane. The Ins(1,4,5)P_3-activated transmembrane channel appears to be identical to the recently described mitogen-regulated, voltage-insensitive Ca^{2+} permeable channel involved in T-cell activation[18]. We suggest that Ins(1,4,5)P_3 acts as the second messenger mediating transmembrane Ca^{2+} influx through specific Ca^{2+}-permeable channels in mitogen-stimulated T-cell activation.

Single-channel currents were recorded from excised inside-out patches of the Jurkat E6-1 human T-cell line[19] by the extracellular patch clamp technique[20]. In general, recording pipettes contained isotonic, tetrodotoxin(TTX)-supplemented $BaCl_2$, though similar results were obtained with 'physiological' external solution ($[Ca^{2+}]_o = 2$ mM). Ba^{2+} was chosen as the current carrier over Ca^{2+} because Ba^{2+} blocks the K^+ currents present in T-cell membranes[21–23], and because Ba^{2+} has been shown to be more permeant, and thus improve signal resolution through traditional Ca^{2+} channels[24–27]. In all cases, the bath contained high KCl (150 mM), low Ca^{2+} (10^{-7} M) solution to mimic internal ionic composition.

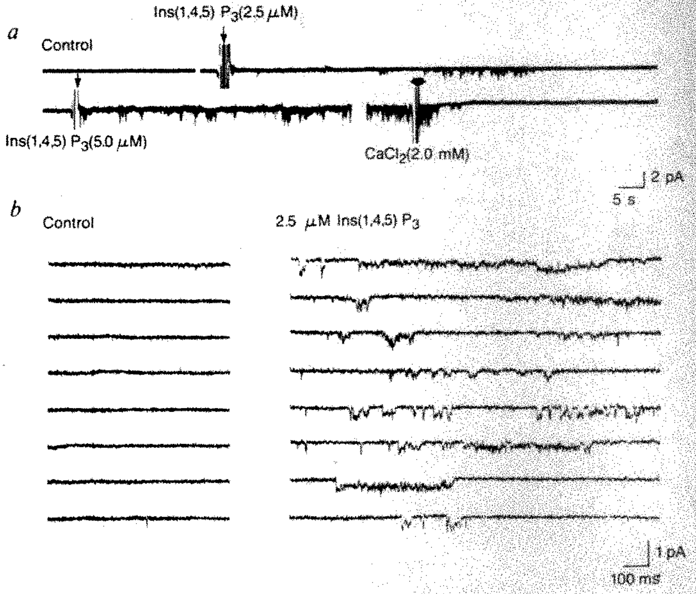

Fig. 1 Single Ins(1,4,5)P_3-induced inward currents recorded from an inside-out membrane patch excised from a Jurkat cell. The patch pipette contained 110 mM $BaCl_2$, 200 nM TTX, and 10 mM HEPES-KOH (*p*H 7.3). The membrane patch cytoplasmic surface was bathed in a solution containing: 150 mM KCl, 0.55 mM $CaCl_2$, 2 mM $MgCl_2$, 1.1 mM EGTA, and 10 mM HEPES-KOH (*p*H 7.3). The free internal Ca^{2+} concentration was estimated at 10^{-7} M using an apparent dissociation constant of 10^{-7} (ref. 48). The holding potential was 0 mV. Inward currents are seen as downward deflections from baseline. All records were filtered at 1 kHz (8-pole Bessel). *a*, Ins(1,4,5)P_3-induced inward currents from one excised inside-out patch at slow sweep speed. Arrows, addition of Ins(1,4,5)P_3 (Sigma) and $CaCl^2$ (final concentrations in parentheses) to the bath. The ringing transients during applications are secondary to vibration. No inward currents were observed in the patch for >5 min before addition of Ins(1,4,5)P_3. The first break in the recording indicates an interruption of 4 min during which the potential was changed. The first bath application of Ins(1,4,5)P_3 resulted in inward current activity within 10 s of addition to the bath. Inward currents appeared <60 s and then disappeared for a period of >4 min. Upper and lower traces were interrupted during this interval. The second application of Ins(1,4,5)P_3 (final concentration 5.0 μM) immediately elicited inward currents which were sustained for longer than 3 min. The interruption in the trace indicates a 2-min interval during which the membrane potential was changed. The inward currents were abolished by the application of $CaCl_2$ (final concentration 2.0 mM) to the internal bathing solution. Current amplitudes were slightly attenuated owing to the limited bandwidth of the pen recorder (Gould 2400S). *b*, Ins(1,4,5)P_3-induced unitary inward currents at higher time resolution with a sampling rate of 4 kHz. Left sequential traces, control recordings before the applications of Ins(1,4,5)P_3. Right traces, inward current traces in the presence of 2.5 μM Ins(1,4,5)P_3. Right traces were not consecutive in time, but selected to illustrate features of inward currents associated with Ins(1,4,5)P_3 (see text). **Methods.** All experiments were performed on cells of the malignant T-lymphocyte cell line Jurkat E6-1, which were maintained in medium RPMI-1640, supplemented with 10% heat-inactivated fetal calf serum, 50 μg ml^{-1} gentamycin, 2 mM L-glutamine (Gibco), at 37 °C in humidified 5% CO_2 atmosphere. Before recording, the cells were washed twice and resuspended in the bathing solution listed above. All experiments were performed at room temperature (20–22 °C). Single-channel currents were recorded using the cell-attached and inside-out recording configurations[20] with a List-EPC7 patch-clamp amplifier. The Sylgaard-coated patch pipettes were pulled from thin-walled borosilicate glass pipettes to a lumen diameter of ≤0.5 μM. Current signals were stored on analogue tape and digitized for analysis.

Figure 1*a* shows the current recorded from one excised patch held at 0 mV. Within seconds after addition of Ins(1,4,5)P_3 (final

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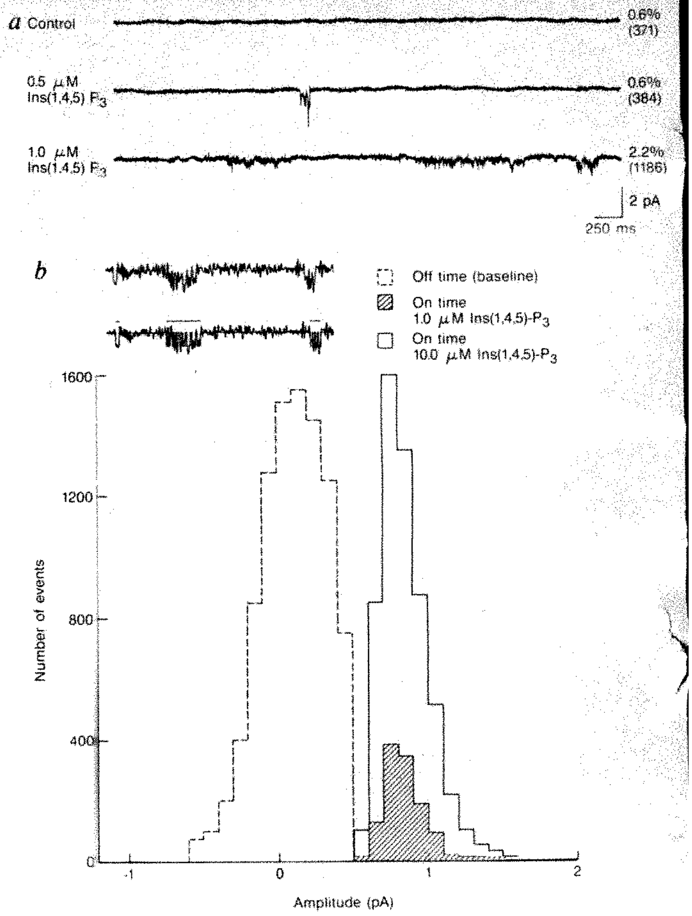

Fig. 2 Effect of $Ins(1,4,5)P_3$ concentration on single channel inward current activity. *a*, Inward current traces from the same inside-out patch in control internal solution and internal solution supplemented with 0.5 or 1.0 μM $Ins(1,4,5)P_3$ as indicated. Numbers at right indicate percent open-time (total open-time over total duration of record) and total number of openings, in parentheses, in 62.259-second continuous recordings under each condition. Recording conditions are as in Fig. 1. *b*, Probability density histogram of current amplitude in the presence of 1.0 μM $Ins(1,4,5)P_3$ (cross-hatched area) and 10.0 μM $Ins(1,4,5)P_3$ (continuous profile). Recording at top left is representative digitized data before and after superposition of computer-derived idealized trace of baseline and singles. (Lines above trace indicate computer-derived bursts, see for example Fig. 3c). The dashed profile indicates the off-time (baseline, corresponding to 0 pA) distribution in the presence of 10.0 μM $Ins(1,4,5)P_3$. The off-time distribution in the presence of 1.0 μM $Ins(1,4,5)P_3$ was similar, but with smaller area, as the number of off events is simply $(n-1)$ open events. For both 1.0 and 10.0 μM concentrations, on-time distributions were fitted from 0.50 pA, the lower single-event detection threshold, which was approximately 2.5 times the mean deviation of baseline noise (computer-derived determination of 0.2068 pA). Period for analysis was 32.768 s. The total number of detected events were 652 for 1.0 μM and 2365 for 10.0 μM. The peak of the on-time amplitude histograms corresponded to a unitary amplitude of 0.71 ± 0.11 pA for 1.0 μM $Ins(1,4,5)P_3$ and 0.71 ± 0.14 pA for 10.0 μM $Ins(1,4,5)P_3$. The area ratio under the two on-time amplitude histograms was 5.0 (5,597 events-pA: 1,117 events-pA), indicating a greater number of events at the higher concentration.

Methods. For data analysis, records on analogue tape were digitized at 10 kHz and stored on a microcomputer (IBM PC-AT). The automated single-channel analysis program of Sachs *et al.*[49] implemented by C. Lingle (personal communication), was used to produce the plots shown in *b* and in Fig. 3*b* and *c* and to calculate kinetics in Table 1. The baseline level (corresponding to 0 pA) was located using an algorithm that maximizes the number of crossings through a horizontal line. An event detection threshold was set at 0.5 times the predetermined single channel amplitude (by eye, which was slightly higher than the computer-derived mean owing to inclusion of short events which do not reach full amplitude), which was confirmed to be 2.5–3.0 times the computer-derived mean baseline noise in each case. Times of transition were defined as crossings of the midline between the baseline and open current level. Multiple event exclusion threshold was set at 1.8 times the predetermined single-channel amplitude. Interval analysis was used to generate distributions of open times, closed times, and burst durations (as in Fig. 3), which were fitted with probability density functions by the method of maximum likelihood to the continuous distribution of data. The first five bins (500 μs) of the open-time and short closed-time distributions were excluded from curve-fitting because of error arising from events inadequately resolved owing to bandwidth attenuation. Patches used for kinetic analysis were selected to contain few, if any, multiple events.

concentration 2.5 μM) to the bath, inward currents appeared. At low concentrations (1.0–2.5 μM), the effect of $Ins(1,4,5)P_3$ appeared to be transient, usually lasting 10–60 s. A second addition of $Ins(1,4,5)P_3$ (final concentration 5.0 μM) promptly elicited sustained inward current pulses. The inward currents abruptly disappeared after addition of millimolar concentrations of Ca^{2+} to the bath, indicating that the currents were not secondary to patch membrane deterioration and suggesting a possible cytosolic Ca^{2+} inactivation process. Figure 1*b* shows the single-channel inward current recorded from another patch at higher time resolution. Addition of 2.5 μM $Ins(1,4,5)P_3$ elicited inward currents, which consisted of intermittent bursts of brief openings, separated by quiet intervals. Unitary current amplitude was ~0.6 pA at 0 mV and slope conductance approximately 7 pS (derived from single-channel *I–V* relationship, not shown).

Similar results were obtained in 14 patches. In 3 of these, inward currents were observed only after the addition of $Ins(1,4,5)P_3$ to the bath, despite observation periods of longer than 5 min for the control period. In 11 other patches, $Ins(1,4,5)P_3$ greatly augmented the inward currents. In all cases, subsequent addition of micromolar or greater Ca^{2+} concentrations to the bath appeared to inactivate the $Ins(1,4,5)P_3$-activated inward currents. Evidence that suggested that the $Ins(1,4,5)P_3$-activated inward current flowed through Ca^{2+}-permeable channels included the following: (1) Ba^{2+} was the only charge carrier with an inwardly directed driving force at 0 mV in the TTX-containing, Na^+-free pipette solution; (2) The amplitude of the single-channel current decreased with increasing depolarization from 0 mV, with no clear-cut reversal identified, indicating a positive reversal potential E_{rev}.

Estimates of the concentration that $Ins(1,4,5)P_3$ can reach in stimulated cells, obtained from direct measurement of labelled $Ins(1,4,5)P_3$, fall in the range 3–75 μM in a variety of cells[3,28–31]. The dependence of channel activation on micromolar $Ins(1,4,5)P_3$ concentrations, which are in the apparent physiological range, was investigated. Activation, observed at threshold concentrations between 0.5 and 1.0 μM (Fig. 2*a*), was dose-dependent at least up to 10 μM. As concentration increased from 1.0 μM to higher concentrations, increasing augmentation of inward current was seen. Augmentation did not occur through a change in unitary current amplitude, but through an apparent increase in frequency of single-channel events. Figure 2 shows the amplitude histogram from one patch measured for 33 s after applications of 1.0 μM and 10.0 μM $Ins(1,4,5)P_3$, respectively. A greater number of opening events were detected at the higher concentration ($n = 652$ for 1.0 μM and $n = 2365$ for 10.0 μM) and the area under the probability distribution curve was correspondingly five times greater, but mean amplitudes of unitary current (0.71 ± 0.11 pA for 1.0 μM and 0.71 ± 0.14 pA for 10.0 μM, mean ± s.d.) were virtually identical. From similar examples, we conclude that $Ins(1,4,5)P_3$ augments inward Ba^{2+} currents with increasing concentrations at least in part by increasing the probability of channel opening.

We have recently demonstrated a voltage-insensitive, Ca^{2+}-permeable channel in the membrane of T lymphocytes[18] which has an increased probability of opening following stimulation

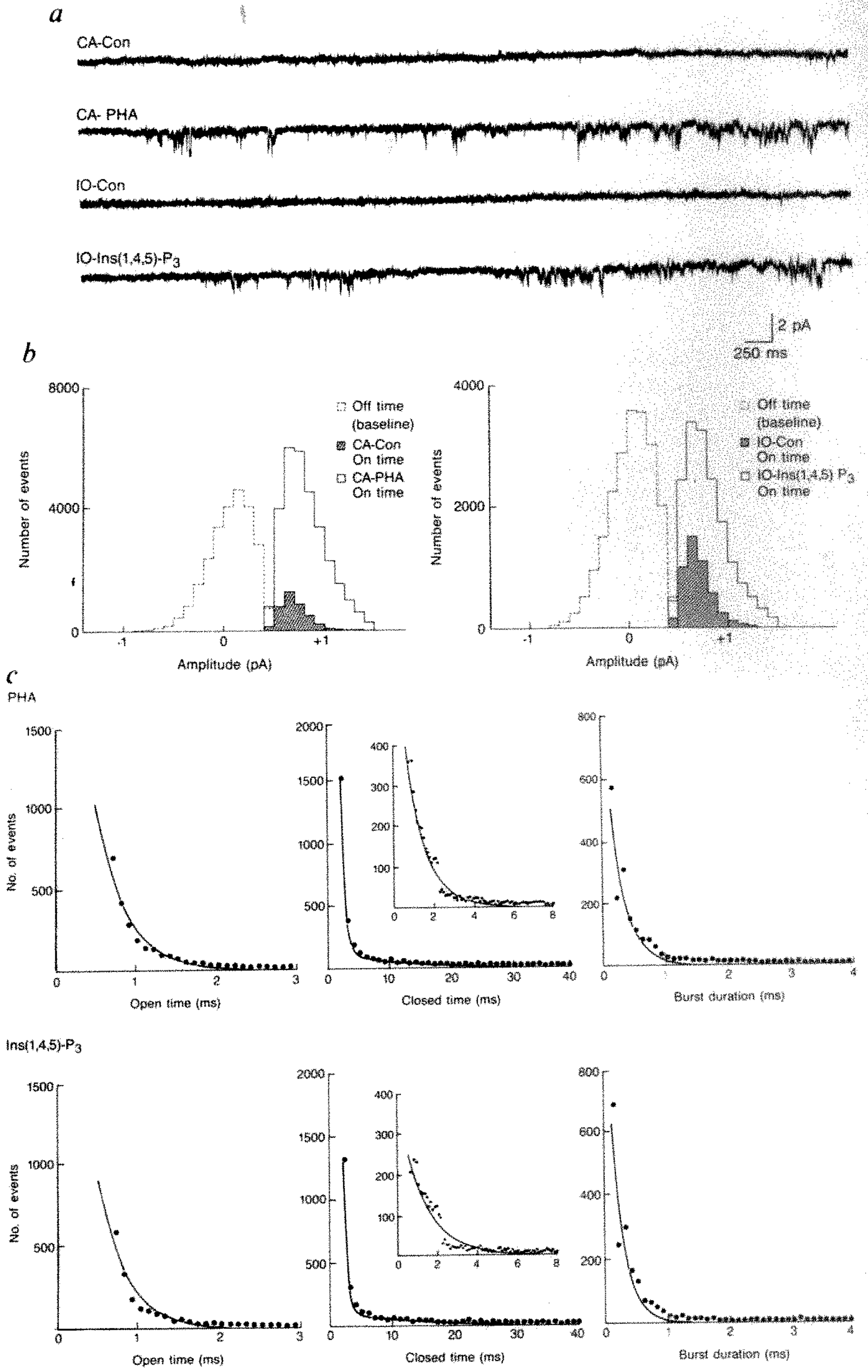

Fig. 3 Comparison of PHA- and Ins(1,4,5)P_3-induced single channel currents. Recordings were made in the cell-attached and inside-out configuration. Pipette solution contained 110 mM $BaCl_2$, 200 nM TTX, and 10 mM HEPES-KOH (*p*H 7.3). Bath solution contained 150 mM KCl, 1 mM $MgCl_2$, 0.55 mM $CaCl_2$, 1.1 mM EGTA, and 10 mM HEPES-KOH (*p*H 7.2). The holding potential was 0 mV in all recordings (cellular resting potential was assumed to be zeroed by the isotonic KCl bath solution). Current signals were filtered at 1 kHz. All records are from the same patch. Probability density histograms were constructed from recordings of 62.259 s for each condition. *a*, Inward currents from a cell-attached patch before (CA-Con) and 5 min after bath application of 6 μg ml^{-1} PHA (CA-PHA), and from the patch isolated into the inside-out configuration before (IO-Con), and 20 s after bath application of 2.5 μM Ins(1,4,5)P_3 (IO-Ins(1,4,5)P_3). Inward currents are seen as downward deflections from baseline. *b*, Probability density histograms of on-time current amplitudes from the patch in the cell-attached configuration before (cross-hatched area) and after (continuous profile) PHA application are in the left panel. On-time amplitude histograms are fitted from 0.4 pA, the lower limit of single event detection threshold, which was >3.0 times the computer-derived mean deviation of baseline noise of 0.13 pA. Dashed line, off-time amplitude (baseline) distribution in the CA-PHA recording. The peak of the on-time amplitude histograms corresponded to a unitary amplitude of 0.57 ± 0.12 pA in the control cell-attached recording and 0.65 ± 0.19 pA after PHA application. The area ratio of PHA to control on-time distributions was 7.9 (28,660 events-pA: 3,620 events-pA). Probability density histograms of on-time current amplitudes from the same patch after isolation into the inside-out configuration before (cross-hatched area) and after (continuous profile) addition of 2.5 μM Ins(1,4,5)P_3 to the bath solution are in the right panel. The dashed line represents the off-time amplitude (baseline) distribution in the IO-Ins(1,4,5)P_3 inside-out recording. The peak of the on-time amplitude histograms corresponded to a unitary amplitude of 0.57 ± 0.12 pA in the control inside-out patch recording, and 0.63 ± 0.18 pA after bath application of Ins(1,4,5)P_3. The area ratio of Ins(1,4,5)P_3 to control was 3.5 (15,530 events-pA: 4,456 events-pA). *c*, Distributions of open times, closed times, and burst durations for the PHA-induced (upper panels) and Ins(1,4,5)P_3-activated (lower panel) inward currents. The durations of the two records analysed were 62.259 s, with a total of 5,689 openings (CA-PHA) and 4,659 openings (IO-Ins(1,4,5)P_3). The open-time and burst duration histograms were fitted with one exponential, and the closed-time distributions were fitted with the sum of two exponentials. The inset above the closed-time distribution represents the same data and fitted curve plotted over a time interval of 0.5–8.0 ms. The time constants (τ) were as follows: for PHA open time, 0.37 ms; closed time 0.6 ms (inset) and 9.9 ms; burst duration, 2.6 ms. For Ins(1,4,5)P_3 open time 0.34 ms; closed time 0.6 ms (inset) and 11.5 ms; burst duration 2.1 ms.

of the T cell by mitogen. Results suggested that stimulation apparently occurs through the mediation of an internal second messenger. The mitogen-operated channel has properties in common with the Ins(1,4,5)P_3-activated conductance, including permeability to Ca^{2+} and Ba^{2+} ions, low single channel conductance and positive E_{rev}, kinetic behaviour characterized as brief openings in bursts, and inactivation by internal Ca^{2+} concentrations $\geq 10^{-6}$ M. To clarify whether Ins(1,4,5)P_3 may serve as the internal second messenger regulating transmembrane Ca^{2+} flux following mitogen stimulation, we compared the inward currents induced by the mitogen phytohaemagglutinin (PHA) in a cell-attached patch, to inward currents activated by Ins(1,4,5)P_3 in the same patch after isolation to the inside-out recording configuration.

The results are shown in Fig. 3. Single-channel records from one patch, (1) cell-attached, before and after bath application of PHA, and (2) inside-out, before and after bath application of Ins(1,4,5)P_3 are shown in Fig. 3*a*. The cell was suspended in isotonic KCl, so transmembrane patch potential was assumed to be 0 mV in both the cell-attached and inside-out configurations. The similar appearance of the PHA-elicited and Ins(1,4,5)P_3-activated conductances, characterized as intermittent bursts of brief openings with low amplitudes, suggested that they were the same channel. Comparative quantitative analysis confirmed this impression. Probability density histograms of unitary current amplitude (Fig. 3b) were virtually identical. In each case, interval analysis revealed monoexponentially distributed open times and burst durations, and double exponentially distributed closed times; the kinetic parameters derived from open-time, closed-time and burst duration histograms were similar. Kinetic parameters are presented for comparative purposes only, as the limited signal-to-noise ratio precludes accurate quantitative kinetic analysis. These results strongly suggest that the Ins(1,4,5)P_3-activated channel is the same as the one turned on during PHA-induced mitogenesis, and thus that Ins(1,4,5)P_3 is the internal second messenger mediating Ca^{2+} channel activation following mitogen-stimulation of T lymphocytes.

There is experimental precedence. Receptor-mediated activation of T lymphocytes is accompanied by prompt, sustained increases in phosphoinositide turnover[32-37], in Ins(1,4,5)P_3[38] and in free cytosolic Ca^{2+} ions[19,39-42]. Experimental evidence obtained with permeabilized Jurkat cells suggests that the initial, transient increase in Ca^{2+} is due to Ins(1,4,5)P_3-mediated release of Ca^{2+} from intracellular stores[38]. However, both Ins(1,4,5)P_3 and Ca^{2+} continue to rise during the first 10 min after T-cell activation and are still elevated at 30 min. It has previously been shown that the mitogen-regulated Ca^{2+}-permeable channel becomes active as early as 20 s after addition of mitogen and has an augmented opening probability for at least 10 min following T-cell activation. A reasonable hypothesis supported by the experimental data is that T-cell activation by mitogens elicits PtdIns(4,5)P_2 breakdown to the second messenger, Ins(1,4,5)P_3, which then acts both to release internal Ca^{2+} stores for the initial transient increase in Ca^{2+} and to activate the transmembrane Ca^{2+}-permeable channel for more sustained increases in Ca^{2+}. Evidence for Ins(1,4,5)P_3-initiated transmembrane Ca^{2+} flux is also strong in blowfly salivary glands[43,44] and clonal rat pituitary cells[45]. Alternatively, preliminary experiments with rat liver plasma membrane vesicles indicate that inositol 1,3,4,5-tetrakisphosphate (Ins(1,3,4,5)P_4), which is derived by 3-phosphorylation of Ins(1,4,5)P_3 by a soluble kinase[46,47], promotes transmembrane Ca^{2+} fluxes. The generation of inositol polyphosphates which act as second messengers mediating transmembrane Ca^{2+} flux may be a mechanism common to a variety of other cell types exhibiting the phosphatidylinositol turnover response, whereby activation of cell-surface receptors brings about a sustained rise in cytosolic Ca^{2+} concentration.

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HLA class II induction in human islet cells by interferon-γ plus tumour necrosis factor or lymphotoxin

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**HLA class II molecules are surface glycoproteins which are esse
tial in the initiation of immune responses. It has been postulat
that induction of class II in epithelial cells such as endocrine cel
which are normally class II negative[1], may result in autoimmunit
In type I diabetes, islet beta cells, the target of the autoimmu
process, selectively express class II antigens[3,4]. But in contrast
most other cell types[5], islet beta cells are not stimulated to expre
class II by interferon-γ (IFN-γ)[6,7] and thus the conditions und
which this induction occurs have been particularly elusive. T
cytotoxins tumour necrosis factor (TNF) and lymphotoxin (L
synergize with IFN-γ in a number of activities[8]. We report he
that IFN-γ in combination with either TNF or LT induces isl
cell class II expression. This finding has important implicatio
for the pathogenesis of type I diabetes and the understanding
the differential control of class II expression.**

The observations that normal thyroid follicular ce
(thyrocytes) can be induced to express class II antigens[9] a
that these products are spontaneously present in thyrocytes fro
glands afflicted by autoimmune disease (Graves' thyrotoxico
or Hashimoto's thyroiditis)[10] led us to propose a new conce
of the pathogenesis of cell-specific autoimmunity[2]. Accordi

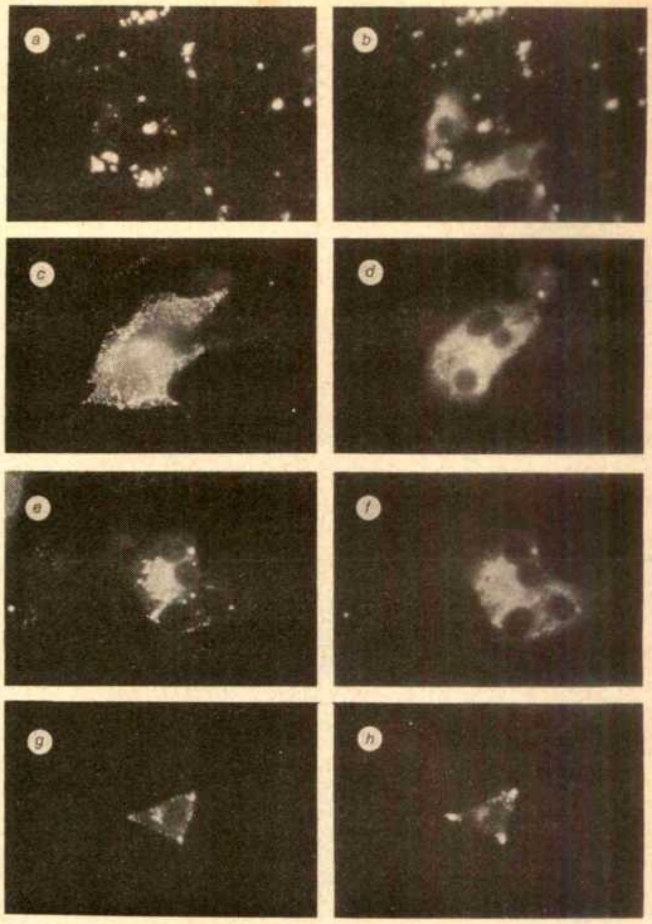

Fig. 1 Indirect immunofluorescence micrographs (×700) of 8-day-old islet-cell-enriched pancreatic cultures stained three days after the addition of IFN-γ (50 U ml^{-1}) and TNF (100 U ml^{-1}). Cells were stained to detect membrane (*a, c, g*) and cytoplasmic (*e*) HLA class II antigens (fluorescein left-hand photography) and C-peptide (*b, d, f*) or glucagon (*h*) (rhodamine right-hand photography). Cultures which were not stimulated with IFN-γ and TNF or LT were negative when stained for class II (*a*) but contained cells expressing C-peptide (*b*). By contrast, in the stimulated cultures (*c–h*), a group of three C-peptide-positive beta cells (*d*) show positive granular surface membrane staining for class II (*c*); of a cluster of four beta cells positive for C-peptide (*f*) two show cytoplasmic class II positive staining (*e*); one isolated alpha cell showing class II membrane staining (*g*) was counterstained by *mAb* to glucagon (*h*).

Methods. Pancreatic cultures, from 8 different glands obtained at surgery from chronic pancreatitis ($n = 2$) and from kidney donors ($n = 6$) were prepared following the method recently described[28] modified when necessary to facilitate the processing of smaller amounts of tissue[6]. After digestion and filtration through meshes of different diameter, aliquots of 10^5 cells were plated on glass coverslips placed in 24-well plates (Nunc) with 0.5 ml of RPMI 1640 culture medium containing 15% fetal calf serum (FCS) (Sera Lab), 2 mM glutamine, 5 mg ml^{-1} transferrin (Sigma) and 10^{-8} M hydrocortisone (Sigma). At day 3 to 6 the medium was changed, FCS concentration lowered to 5% and the different supplements such as IFN-γ, TNF and LT added. IFN-γ (specific activity $\sim 3 \times 10^7$ U per mg protein; endotoxin contamination less than 0.125 ng per mg protein) and TNF (specific activity 5×10^7 U per mg protein; endotoxin contamination less than 0.125 ng per mg protein) were prepared from recombinant sources, whereas LT (specific activity $1\text{–}2 \times 10^7$ U per mg protein) was purified from supernatants of RPMI 1788 cells to a final purity of at least 90%[29]. Double immunofluorescent staining of the cell monolayer was carried out 2–8 days after the addition of the supplements following a protocol previously described[6]. After extensive washing in Hanks balanced salt solution containing 1% BSA viable monolayers were first incubated for 30 min at room temperature with immunoglobulin (IgM) mouse mAb RFDR1 to the non-polymorphic region of the class II molecules (G. Janossy, Royal Free Hospital, London) washed again and subsequently stained with fluorescein isothiocyanate (FITC)-labelled affinity-purified goat anti-mouse (IgM) (μ-chain specific, Southern Biotechnology). Cultures were further washed and fixed for 5 min in 50% v/v acetone/methanol to expose cytoplasmic hormonal contents and incubated with the corresponding anti-pancreatic-hormone specific IgG mouse mAb (PEP 030: anti C-peptide: Som 018: anti-somatostatin; Gluc 1: anti-glucagon, all from Dr P. N. Jorgensen, Novo, Copenhagen). TRITC-(tetramethyl rhodamine isothiocyanate) labelled affinity-purified goat anti-mouse IgG (γ-chain specific, Southern Biotechnology) was then used to develop the reaction. Non-specific binding was ruled out by staining parallel cultures with an unrelated mAb of the same immunoglobulin class or normal mouse serum at dilution of 1:500. Undesirable cross-reactivities among reagents from different species were assessed by omitting one of the layers in turn. All preparations were examined with ×63 oil-immersion objective under a fluorescence Zeiss III photomicroscope equipped with epi-illumination.

to this hypothesis, the transition from a local non-specific inflammatory reaction to autoimmunity would occur as a consequence of the aberrant expression of HLA class II molecules by the epithelial endocrine cells. In genetically susceptible individuals these cells could then efficiently present their own surface autoantigens to autoreactive helper T lymphocytes, bypassing the requirement for classical antigen-presenting cells. This concept is strongly supported by our observations that class II-expressing thyrocytes can present exogenous peptide[11] or endogenous autoantigens[12] to T-cell clones. The applicability of this hypothesis to type I diabetes has not yet been ascertained, but is suggested by the occurrence of inappropriate class II expression in the pancreatic islet beta cells of diabetic patients (refs 3 and 4; see ref. 13 for review).

Using the double immunofluorescence technique on monolayer cultures of human pancreas enriched in islet cells we previously showed that IFN-γ induces strong class II expression in pancreatic exocrine and ductal cells but not in the islet beta cells[6]. However, IFN-γ increases class I expression in human beta cells[6,7] demonstrating the presence of IFN-γ receptors on these cells. When human pancreatic cells were similarly cultured with IFN-γ and TNF or IFN-γ and LT for 2–8 days, up to 50% of the beta cells showed positive membrane and cytoplasmic staining for class II, of intensity comparable to that seen in the ductal cells (Fig. 1). IFN-γ at 10 U ml^{-1} was sufficient when used together with 50 U ml^{-1} or more of either of the cytotoxins. Conversely, 10 U ml^{-1} of either cytotoxin was effective in conjunction with doses of IFN-γ of at least 50 U ml^{-1} (Fig. 2).

The HLA class II expression induced *in vitro* on the beta cells was detected with various monoclonal antibodies (mAbs) including DA6.164, specific for the HLA-DR β-chain (Dr K. Cuy, Edinburgh) and VIC-Y1, specific for the cytoplasmic invariant chain (Professor W. Knapp, Vienna). A limited number of alpha and delta cells were identified in the cultures by using the anti-glucagon mAb Gluc-1 and the anti-somatostatin mAb SOM018, in the double immunofluorescence technique: both of these cell types also expressed class II when cultured with either of the combinations of IFN-γ with TNF or LT (Fig. 1*g* and *h*).

Interleukin-1 (IL-1) shares many of the biological effects of TNF[14] and has been shown to influence beta-cell function[15]. We accordingly tested the effect of r(recombinant)IL-1α and rIL-1β (P. Lomedico, Hoffmann LaRoche and C. Lewis, Glaxo) alone or together with IFN-γ on class II expression by islet cells following the same protocol used for TNF. However both types of IL-1 were ineffective in the dose range 10–1,000 U ml^{-1}. The number of beta cells in the monolayers was unaffected by IL-1. The specificity of the effects of the TNF and LT preparations was established by observing >80% reduction in the number of class II-positive beta cells in cultures supplemented with 100 U ml^{-1} of IFN-γ together with TNF or LT pre-incubated with neutralizing antisera (see legend to Fig. 2*c* and *d*).

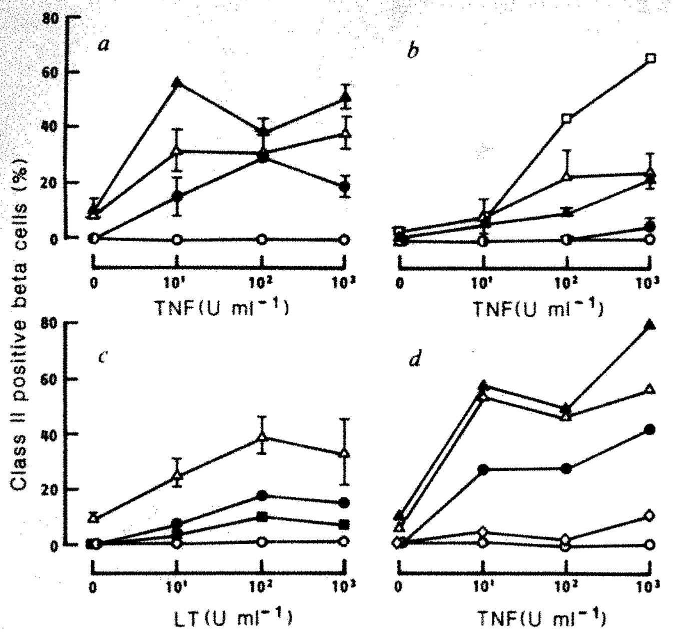

Fig. 2 Synergistic effect of IFN-γ and TNF (*a*, *b*, *d*) or LT (*c*) on beta cell class II expression. Pancreatic cell cultures were simultaneously supplemented at day 2 to 6 and tested 3-7 days later. *a*, *b*, *c*, Mean of data (bars ± s.e.m.) obtained from nine different sets of cultures in which cells from eight different glands were used. *d*, Results from an experiment carried out with cultures from a single pancreas. Graphs *a*, *c*, *d* refer to membrane, and *b* to cytoplasmic, class II expression. Symbols ○, ●, △ and ▲; 0, 10, 100 and 1,000 U ml^{-1} of IFN-γ respectively. In *b*, □ indicates cytoplasmic class II expression as detected by mAb VIC-Y1, directed against the invariant chain of class II molecules. In the absence of IFN-γ neither TNF nor LT induced class II expression in beta cells whereas IFN-γ by itself had a weak effect on a small percentage of beta cells (see text). In *c*, ■ indicates the reduced dose response to 1,000 U ml^{-1} of IFN-γ and increasing doses of LT when these factors were preincubated for 4 h with rabbit antibodies to LT (batch 25C, final neutralizing capacity 1,000 U of LT ml^{-1}). In *d*, ◇ indicates the similarly reduced dose response to 1,000 U ml^{-1} of IFN-γ and increasing doses of TNF when these factors were preincubated with a rabbit antibody to TNF (batch 15-1, final neutralization capacity in the cultures 1,000 U TNF ml^{-1}).

In cultures supplemented with the cytotoxins alone, class II induction was not observed. IFN-γ alone at doses over 100 U ml^{-1} occasionally produced weak class II expression in up to 10% of the beta cells, but the weakness of this staining led us to doubt its significance particularly when compared with that seen on the ductal cells in the same preparations[6] or on human thyroid cultures[5]: others have reached similar conclusions[7]. The interpretation of these results is not clear but one possibility is that islets from certain donors were exposed *in vivo* to TNF or LT, thus rendering them weakly susceptible to induction by IFN-γ *in vitro*. Pancreatic monolayer cultures, though enriched in islet cells, contained over 80% non-endocrine cells among which ductal cells predominate but macrophages, dendritic-like cells and fibroblasts were also present. It is therefore possible that the effect of IFN-γ and TNF, or IFN-γ and LT, on the islet cells is exerted or potentiated through mediator(s) released by other cell types as shown in another system[16]. However, a direct action on the beta cells is favoured by the observation that the intensity of class II induction observed was similar in cultures in which the representation of the different cell types varied widely.

TNF and LT have recently been characterized and cloned[17,18]. They show 30% sequence similarity, use the same receptor and synergize with IFN-γ in their cytotoxic action on transformed or malignant cells[19,20]. Neither TNF nor LT, either alone or together with IFN-γ, seemed to have an acute cytotoxic effect on beta cells as the number of these cells present in control and treated cultures did not differ significantly. The observations reported here constitute the first demonstration that TNF and LT have an influence on major histocompatibility complex (MHC) class II expression in human epithelial cells. These findings have wide implications for understanding how TNF and LT exert their immunomodulatory effects and stress the recently recognized similarities between the spectrum of action of these mediators and that of interleukins and interferons[21-24].

The finding of experimental conditions in which human beta cells can be induced to express HLA class II molecules not only strengthens the previous histopathological observations of the pancreas of diabetic individuals[3,4] but also points to a possible sequence of events leading to this phenomenon *in vivo*. It has recently been shown that either TNF or LT in combination with IFN-γ can protect cells from viral infection although they kill those already infected[8], so it is conceivable that the local release of these mediators in response to a viral infection of the beta cells is one of the mechanisms underlying inappropriate class II expression in the diabetic pancreas. The short half-life of these lymphokines should limit their sphere of action and thereby help to explain the beta cell specificity of the class II expression observed in the diabetic pancreas.

The results reported here demonstrate that multiple signals with a degree of cell and tissue specificity are necessary for the most efficient induction of class II expression. Such differential regulation is most dramatically illustrated with islet cells in which IFN-γ alone is virtually ineffective, but it also applies to other cell types[25-27]. This synergy of mediators may limit the circumstances in which inappropriate induction of class II occurs and explain the marked heterogeneity of class II expression in different cell types. Furthermore, it indicates the possibility of therapeutic intervention in autoimmune diseases by selectively reducing class II expression in particular cell types, and avoiding the generalized immunosuppression that would result if class II expression were abrogated in all antigen-presenting cells.

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Dominance of one T-cell receptor in the H–2K^b/TNP response

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T-cell receptors (TCRs) recognize foreign antigens in the context of major histocompatibility complex (MHC)-encoded cell surface proteins. These receptors are heterogeneous, dimeric glycoproteins composed of disulphide linked α- and β-chains. We analysed the diversity of TCRs in a collection of H–2K^b-restricted, 2,4,6-trinitrophenyl (TNP)-specific (H–2K^b/TNP) cytotoxic T-cell (T_c) clones from C57BL/6 mice. Investigation of the β-chain messenger RNAs revealed that nearly half of these independent clones expressed an identical β-chain gene[1]. We show here that almost all the T_c clones expressing the predominant β-chain gene also express an identical α-chain gene. These results show that a strong selective pressure acted on the T_c population, resulting in a skewing of the TCR repertoire for H–2K^b/TNP and in the dominant expression of one TCR with this specificity. Possible explanations for this skewing include antigen-driven clonal expansion and network interactions.

Vertebrate T cells are capable of recognizing a multitude of foreign antigens in the context of MHC-encoded cell-surface proteins[2]. The diversity of TCR specificities results from the somatic rearrangement of germline gene segments for both α- and β-chains[3]. The same mechanisms for generating immunoglobulin diversity (such as germline diversity, junctional diversity, combinatorial joining and combinatorial association) also generate diversity for TCRs, with the possible exception of somatic mutation[4]. The α- and β-chains of TCR alone confer antigen and MHC specificity on T cells[5]. The antigen and MHC specificities are properties of the TCR as a whole rather than separate properties of the α- and β-chains[5,6]. As yet, no general correlations between TCR structure and function have been observed.

Although the potential number of TCRs is high, the actual number expressed in T-cell populations with a given specificity is unknown[7]. The analogous problem of immunoglobulin repertoire diversity has been studied extensively[8], but the differences between the functions of immunoglobulins and TCRs preclude extension of these results to TCRs. The restriction of the TCR repertoire may reflect specific aspects of T-cell function. For instance, TCRs are selected for restriction to recognizing antigen in the context of one self-MHC encoded protein and for tolerance to all self-MHC encoded proteins[9-11]. Further, possible preferential expansion of some T cells expressing a given specificity or interactions of idiotypic networks might also alter TCR repertoire.

To address the question of the diversity of the TCR repertoire, we have analysed the TCR genes in a panel of 42 independent T_c clones specific for H-2K^b/TNP[12]. Previously we reported that almost half of these clones express identical β-chain genes[1]. The high frequency of this β-chain might result from its ability to combine with a large number of different α-chains to generate H-2K^b/TNP specificity. Alternatively, the β-chain may be associated with only one or a few related α-chains, indicating a strong selection for this TCR idiotype. Paradigms for both possibilities exist in the studies of B-cell repertoire[13,14]. To answer this question we have now analysed the α-chain mRNAs of these T_c clones.

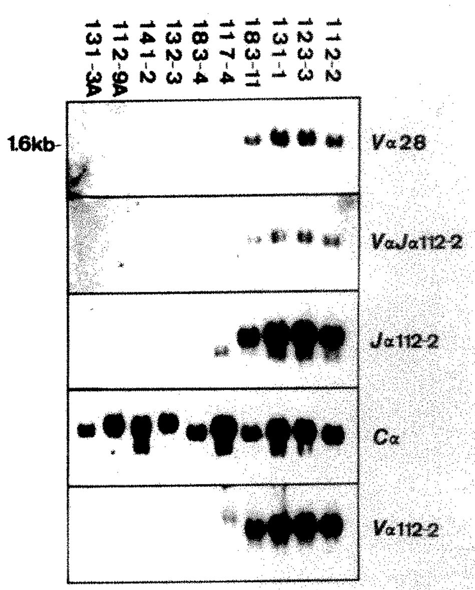

Fig. 2 Analysis of T-cell receptor α-chain mRNAs with oligonucleotide probes. RNA from 10 different T_c clones specific for *H-2K*^{b}/TNP were hybridized with five different ^{32}P-labelled oligonucleotides specific for different TCR gene segments. For description of oligonucleotide probes see Fig. 1 and for details of methods see refs 1, 22 and 23.

We cloned a partial cDNA copy of the α-chain mRNA from one of the T_c clones expressing the predominant β-chain mRNA, T_c clone 112-2. The cDNA was made from total RNA of T_c clone 112-2 and inserted into the bacteriophage vector λgt11. This library was screened with an oligonucleotide probe specific for the constant region of the α-chain gene. Strongly hybridizing clones were isolated and the cDNA inserts sequenced. One clone had an insert of 650 bp which consisted of the 3′ portion of V_α, a J_α and the 5′ portion of C_α (Fig. 1). On the basis of this sequence we synthesized oligonucleotide probes (see Fig. 1). The V_α112-2 probe is complementary to the V_α gene segment expressed in T_c clone 112-2 and two other sequences in the genome (data not shown). The V_α28 probe is complementary to the 3′ end of the V_α112-2 and of the V_α28 gene segments (nomenclature according to ref. 15). The probe specific for J_α112-2 is a previously unreported J_α gene segment expressed in T_c clone 112-2. Finally, the $V_\alpha J_\alpha$112-2 probe is complementary to the junction between the V_α112-2 and J_α112-2 gene segments.

Fig. 1 Nucleotide sequence of the $V_\alpha J_\alpha$ junction in the H-2K^b/TNP-specific T_c clone 112-2 and derived synthetic oligonucleotide probes.

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                    ───────────────────────────────────────V──────────────────────────────────────
cDNA      5' ...TTGAAGCCACATACAATAAAGAAACCACCTCCTTCCACTTGCAGAAAGCCTCAGTGCAAGAGTCAGACTCGGCTGTGT...

Oligo-            3' TCGGTGTATGTTATTTCTTTGGT 5'      3' CGTCTTTCGGAGTCACGTTCTCA 5'
probes                    Vα112-2                                 Vα28

                ───────V────────┼- - - - - - - - - - - - - J - - - - - - - - - - - - - - - - - -┼C─
cDNA            ...ACTACTGTGCTCTGGGTGATCCCTATGCCCAGGGATTAACCTTCGGTCTTGGCACCAGAGTATCTGTGTTTCCCTACA... 3'

Oligo-          3'  TGACACGAGACCCACTAGGGATA 5'          3' AAGCCAGAACCGTGGTCTCATA 5'
probes                   VαJα112-2                                 Jα112-2
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Methods. A cDNA library was constructed from total cellular RNA of T_c clone 112-2 in the bacteriophage vector λgt11[19] as described[1]. Screening was carried out using an oligonucleotide probe (5′ GTGCTGTCCTGAGACCGAGGATC 3′) specific for the C_α region[20]. Three independent positive cDNA phage clones with inserts of 500–650 base pairs were subcloned into pUC12 and M13mp9. Sequence analysis was carried out according to Sanger *et al.*[21] with universal and specific primers. The sequences consist of more than 100 base pairs of the 3′ end of a V_α, J_α and a nearly full-length C_α element.

α-Chain gene element				Designation of T_C clones	β-Chain gene element			
V_α 112-2	V_α 28	$V_\alpha J_\alpha$ 112-2	J_α 112-2		V_β 3	D_β 112-2	J_β 2-6	C_β 2
▨	▨	▨	▨	112-2	▨	▨	▨	▨
▨	▨	▨	▨	112-3	▨	▨	▨	▨
▨	▨	▨	▨	117-3	▨	▨	▨	▨
▨	▨	▨	▨	123-1	▨	▨	▨	▨
▨	▨	▨	▨	123-3	▨	▨	▨	▨
▨	▨	▨	▨	131-1	▨	▨	▨	▨
▨	▨	▨	▨	147-3	▨	▨	▨	▨
▨	▨	▨	▨	162-1	▨	▨	▨	▨
▨	▨	▨	▨	163-2	▨	▨	▨	▨
▨	▨	▨	▨	177-1	▨	▨	▨	▨
▨	▨	▨	▨	182-5	▨	▨	▨	▨
▨	▨	▨	▨	183-2	▨	▨	▨	▨
▨	▨	▨	▨	183-6	▨	▨	▨	▨
▨	▨	▨	▨	183-9	▨	▨	▨	▨
▨	▨	▨	▨	183-11	▨	▨	▨	▨
▨	▨	▨	▨	187-2	▨	▨	▨	▨
*	▨	*	▨	122-1	▨	▨	▨	▨
*	▨	*	▨	122-6	▨	▨	▨	▨
*	▨			133-2	▨	▨	▨	▨
				112-6A			▨	▨
▨	▨			117-1			▨	▨
				117-4			▨	▨
			▨	131-2			▨	▨
				132-4			▨	▨
				137-1			▨	▨
				183-3			▨	▨
				183-3.1			▨	▨
				183-4			▨	▨
				183-7			▨	▨
				132-3				▨
				141-2				▨
				161-1				▨
				163-4				▨
				183-1				▨
				112-9A				
				113-4A				
▨				117-2A				
				121-1				
				131-3A				
				142-2A				
				143-2A				
▨				147-1				

Fig. 3 T-cell receptor α- and β-chain genes of H-2K^b/TNP-specific T_c clones. T_c clones are listed according to their gene structure as determined by Northern blot or RNA or DNA gel hybridizations with the oligonucleotide probes. For detailed description of the clones see refs 1 and 12. From three T_c clones (122-1, 122-6, 133-2) neither RNA nor DNA was available to complete the results for hybridization with the V_α112-2 and the $V_\alpha J_\alpha$112-2 probes. *, Not tested.

These oligonucleotides were used to probe the RNAs of the panel of T_c clones, either by Northern blotting or by direct gel hybridizations. In Fig. 2 we show a representative example of the hybridization results; the results of all the RNA hybridizations are summarized in Fig. 3. With one exception (T_c clone 133-2) all the T_c clones expressing the predominant β-chain mRNA express α-chain mRNAs composed of identical germline segments. All the rearrangements of V_α112-2 to J_α112-2 generated very similar, probably identical, $V_\alpha J_\alpha$ junctions as evidenced by the hybridization of the $V_\alpha J_\alpha$112-2 probe. Eight of the sixteen T_c clones expressing this αβ dimer were shown to be independent on the basis of having different γ-chain or non-productive β-chain rearrangements (T_c clone 112-2, 117-3, 123-3, 131-1, 147-3, 177-1, 183-2, 183-6; data not shown). Thus these T_c clones are not sister isolates but rather independent examples of identical α- and β-chain gene rearrangements. Of the T_c clones not expressing the predominant β-chain mRNA, one T_c clone (117-1) expresses an α-chain mRNA composed of V_α112-2 fused to another J_α and T_c clone 131-2 has a different V_α fused to J_α112-2. Finally, two T_c clones (117-2A, 147-1) express mRNA which hybridizes with the V_α112-2 probe but not with the V_α28 probe and thus express a related V_α element.

The oligonucleotide probes were also used in hybridizations with B6 liver and T_c clone DNAs. As expected, the oligonucleotide probe specific for the junction $V_\alpha J_\alpha$112-2 does not hybridize to the germline DNA but only to DNA of those T_c clones expressing the V_α112-2/J_α112-2 mRNA. The migration of the $V_\alpha J_\alpha$112-2 hybridizing band is coincident with the migration of a V_α112-2 and a J_α112-2 hybridizing non-germline band in these T_c clones (data not shown). Although both V_α112-2 and V_α28 probes are complementary to multiple fragments in liver DNA in all 16 T_c clones expressing the same V_α gene the identical V_α element is rearranged.

As the sequence of V_α112-2 has not been published, we isolated the B6 liver genomic fragment containing V_α112-2. The sequence is shown in Fig. 4: V_α112-2 has similarity with the V_α4 family[15] (versus V_α28: 79% in the nucleic acid sequence and 77% in the predicted protein sequence; versus V_α65: 78% in the nucleic acid sequence and 73% in the predicted protein sequence).

In summary, 40% (16/42) of independent T_c clones isolated for H-2K^b/TNP specificity express α- and β-chain genes composed of identical germline segments and with identical joints. This result has two significant aspects: that one TCR can dominate the immune response and that the sequence of this TCR is highly conserved in independent isolates. The high degree of sequence conservation is surprising in the light of the work of Fink *et al.*[4]. In analysing five T helper-cell clones specific for *H-2IE*^{k}/pigeon cytochrome *c*, they found that the TCRs were related, but varied significantly in D_β, J_β and J_α sequences. These differences correlated with altered antigen cross-reactivity and alloreactivity. The findings suggest that in this system significant variation in α- and β-chain genes can still result in the same antigen/MHC specificity. Our own finding that the predominant β-chain may complex with either the predominant α-chain or with a related α-chain (T_c clone 133-2) to confer the same specificity support this view. Thus we suspect that the conservation of the predominant α- and β-chain gene sequences in our system is not required for antigen/MHC specificity, but reflects its selection at one or several stages in T-cell development.

For T cells expressing one TCR to dominate the response to a given antigen/MHC combination, they must have had a selec

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CTGCAGGCTCACAATAGATGCCAGGCATATAAAGGTGTGTGTTATTTCTATGTATGCTTAGAGCTGTAATCTGAAGGAAAACAACCTTGAGATGCTTTCC
                                                                                                   100
GTTTAAAGTGCAGACAGTGTCTCTCTATGTTGGAACTCTATTTGTAAAGCAGGATAGTTCCAAAATCACAGAGCTATACCTTGATAGCTGTCTTTGGACA
                                                                                                   200
TAAAGGACTGAAAGACTGACATACACTTTATTGGTAAGATCAGGGAGAGAGCCCACTGAAATATCCATCCTCAGTTCAGCTCCTGTCCACCAGGGGGCGC
                                                                                                   300
AGATGCCTAACACTGGCTTGTTTCCTGGGTGGTTTATGTCTAAGATGACTCACAGTTTCTCCGAGTTAACACTCCTTCATGCTAAGCATCAAGACCACTT
                                                                                                   400
      M  T  L  K  M  D  S  S  P  G  F  V  A  V  I  L  L  I  L
TCTAGATGACACTAAAGATGGACTCTTCTCCAGGCTTCGTGGCTGTGATACTTCTCATACTTGGTAAGTAAACAGAGTATTCTTTAGCGGTAAGTTATCT
                                                                                                   500
                                                                        D  S  V  T  Q  T  E  G  P  V
TCAAGGCAACACAGAGCTTATCTCCCTCTTCCTTTCTCCCTTCCCTTTGCACTGTAGGAAGGACCCACGGAGATTCCGTGACTCAAACAGAAGGCCCAGT
                                                                                                   600
  T  V  S  E  S  E  S  L  I  I  N  C  T  Y  S  A  T  S  I  A  Y  P  N  L  F  W  Y  V  R  Y  P  G  E
GACCGTCTCAGAAAGCGAGTCCCTGATAATAAATTGCACGTATTCAGCCACAAGCATAGCTTACCCTAATCTTTTCTGGTATGTTCGATATCCTGGAGAA
                                                                                                   700
 G  L  Q  L  L  L  K  V  I  T  A  G  Q  K  G  S  S  R  G  F  E  A  T  Y  N  K  E  T  T  S  F  H  L
GGTCTACAACTCCTCCTGAAAGTCATTACGGCTGGCCAGAAGGGAAGCAGCAGAGGGTTTGAAGCCACATACAATAAAGAAACCACCTCCTTCCACTTGC
                                                                                                   800
Q  K  A  S  V  Q  E  S  D  S  A  V  Y  Y  C  A  L  G
AGAAAGCCTCAGTGCAAGAGTCAGACTCGGCTGTGTACTACTGTGCTCTGGGTGACACAGTGGCAGAAACTGCAG
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Fig. 4 Nucleotide sequence of the unrearranged V_α112-2 gene segment. The predicted amino-acid sequence encoded by the leader and the V region exons is identified by the single letter code. A library of B6 DNA in Charon 28[24] was screened with V_α112-2 and V_α28 oligonucleotide probes. Positive clones were isolated, subcloned into M13mp9, and sequenced according to Sanger *et al.*[21].

tive growth advantage over other T cells with the same specificity. This advantage might be expressed at a number of stages in T-cell development. The rearrangement of TCR germline segments may not be random. An example of non-random rearrangement exists in the choice of immunoglobulin *V* gene segments early in B-cell ontogeny[16]. The selection for self-MHC restriction and tolerance may favour T cells expressing certain receptors[9-11]. During the immune response itself the stimulation with antigen may preferentially expand the population of T cells expressing certain TCRs. The basis for this preferential stimulation need not be higher affinity as is seen in the maturation of B-cell responses[8]. Finally, the TCR may confer an advantage in the context of an idiotypic network[17]. Indeed, T cells expressing anti-TCR idiotype specificity have been isolated[18].

Our study shows that a significant skewing of the TCR repertoire occurs in the response to H-2K^b/TNP. Further work will be necessary to determine the generality of dominant TCRs in T-cell responses and the exact nature of the selection for these TCRs.

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Possible role for fatty acyl-coenzyme A in intracellular protein transport

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The transport of proteins between subcellular compartments is a vectorial, energy-requiring process mediated by the budding and fusion of a series of vesicular carriers[1]. As yet, nothing is known of the chemical reactions that underlie these events, or how or in exactly what forms energy is used to sustain such movements. Here we report that fatty acyl-CoA acts as cofactor to a Golgi-associated protein factor (termed NSF) that is required for transport between cisternae of the Golgi stack in a cell-free system. This previously unsuspected connection may offer a link between the complex process of protein transport and a single, well-defined type of chemical reaction. We suggest that an ATP-dependent cycle of fatty acylation and deacylation may play an important role in driving rounds of vectorial protein transport.

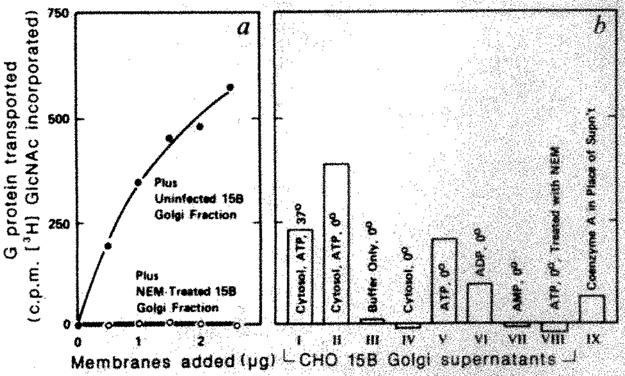

Fig. 1 Restoration of transport activity of NEM-treated donor and acceptor by CHO 15B Golgi fraction. *a*, G protein transport with (○) NEM-treated or (●) untreated uninfected CHO 15B Golgi fraction added to assay mixture, in which donor and acceptor had both been treated with NEM, before incubation at 37 °C. *b*, G protein transport in the same assay mixture, containing supernatants of CHO 15B Golgi fraction that had been incubated with various combinations of CHO 15B cytosol and nucleotides at 37 °C (I) or 0 °C (II–VIII). In mixture IX, CoASH replaced CHO 15B Golgi fraction supernatant.

Methods. G protein transport assays were performed as described previously[3]. Reaction mixes (50 μl total volume) contained donor and acceptor Golgi membranes, gel-filtered CHO 15B cytosol, 50 μM ATP (standard concentration), an ATP-regenerating system, and assay buffer consisting of 25 mM HEPES-KOH (*p*H 7.0), 15 mM KCl, and 2.5 mM magnesium acetate. Incubations were for 60 min at 37 °C. Cytosol was prespun 30 min at 100,000*g* in a Beckman Airfuge to eliminate any membrane contaminants. NEM was freshly dissolved to a concentration of 50 mM, and diluted 1:50 into Golgi fractions containing 1.0 M sucrose, 10 mM Tris (*p*H 7.4). After 15 min at 0 °C, an equal volume of 100 mM dithiothreitol (DTT) was added to quench unreacted NEM. Immunoprecipitation of G protein was carried out overnight at 4 °C, as DTT inhibited immune complex formation at 37 °C. Protein was measured according to Lowry[19] using bovine serum albumin (BSA) as a standard. In *a*, background incorporation of 143 c.p.m. (obtained in the absence of uninfected 15B Golgi) has been subtracted. For comparison, 787 c.p.m. above background were incorporated into G protein when NEM-treated donor was assayed together with untreated acceptor. Uninfected CHO 15B Golgi were pretreated with NEM (1 mM) by incubating for 15 min at 0 °C followed by 2 mM DTT before addition. In *b*, uninfected CHO 15B membranes (0.42 mg ml^{-1}) were diluted fivefold into assay buffer. Where indicated, 1.6 mg ml^{-1} CHO 15B cytosol and/or 100 μM nucleotide were also present. Sample I was incubated 15 min at 37 °C; all others were kept on ice. Samples (100 μl each) were spun for 10 min at 400,000*g* in a Beckman TL-100 tabletop ultracentrifuge. Samples V and VIII were identical except that the supernatant from sample VIII was treated with NEM (1 mM, 15 min at 0 °C, followed by 2 mM DTT). Portions (25 μl) of the supernatant were assayed for restorative activity. Sample IX contained CoASH (10 μM final concentration) in place of an uninfected 15B membrane supernatant. A background of 223 c.p.m. (with no uninfected 15B supernatant present) has been subtracted. Sample II (25 μl) before centrifugation (containing 2.1 μg CHO 15B membranes) gave 1,076 c.p.m. above background.

The combined requirements for NSF and fatty acyl-coenzyme A were revealed by their ability to restore transport in a cell-free system whose membrane components had been selectively inactivated with the sulphydryl reagent *N*-ethylmaleimide (NEM). This cell-free system reconstitutes the transport of the vesicular stomatitis virus (VSV)-encoded glycoprotein (G protein) between successive cisternae of the Golgi stack[2-7]. This transport reaction requires ATP as well as a cytosol (high-speed supernatant) fraction. G protein starts out in a Golgi-enriched 'donor' fraction from a VSV-infected mutant CHO cell line (clone 15B) that lacks the enzyme *N*-acetylglucosaminyltransferase I. This Golgi fraction is incubated with a comparable Golgi fraction (termed 'acceptor') prepared from uninfected

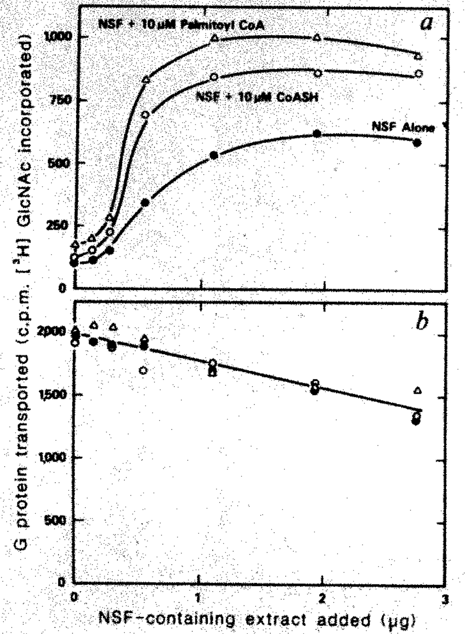

Fig. 2 Stimulation by CoASH and palmitoyl-CoA of the restorative activity of NSF under standard assay conditions. Donor and acceptor membranes were either (*a*) treated with NEM (1 mM, 15 min at 0 °C, followed by 2 mM DTT), or (*b*) not treated. ●, NSF alone; ○, NSF plus 10 µM CoASH; △, NSF plus 10 µM palmitoyl-CoA. Transport assays were performed as in Fig. 1, except the amounts of the components added were reduced by half to give a reaction volume of 25 µl. The NSF-containing fraction was prepared by adding 4.75 ml of uninfected CHO 15B membranes (0.82 mg ml^{-1}) to 19 ml of ice-cold assay buffer containing 100 µM ATP (final concentration). Membranes were pelleted 60 min at 300,000*g* in a Beckman SW-50.1 rotor. The supernatant was concentrated 14-fold using Amicon Centricon 30 microconcentrators, and stored frozen at −80 °C. The active concentrate contained 0.55 mg ml^{-1} protein (measured according to Bradford[20], using bovine serum albumin (BSA) as a standard). CoASH and palmitoyl-CoA (Sigma) were added from aqueous stock solutions.

wild-type CHO cells. When G protein is transported to the *N*-acetylglucosamine-transferring (GlcNAc transferase-containing) *medial*[8] cisternae of the wild-type acceptor Golgi stacks, in an incubation containing UDP-[^{3}H]GlcNAc, then [^{3}H]GlcNAc is incorporated into G protein, providing a measure of transport during the cell-free incubation. Current evidence suggests that this transport is mediated by a population of non-clathrin coated vesicles[6].

Pretreatment of both donor and acceptor membrane fractions with NEM (1 mM for 15 min at 0 °C) completely inhibited the transport reaction in assays containing untreated cytosol[9]. However, transport was virtually normal if only the donor or the acceptor fraction was pretreated. This suggested that an NEM-sensitive factor (NSF) was needed for transport, and that NSF could be supplied by either the donor or the acceptor Golgi membranes, each of which would be expected to contain this factor. Because the requirement for NSF was evident in the presence of untreated cytosol, NSF activity cannot be present in appreciable amounts in the cytosol fraction as prepared.

To test this interpretation, we examined whether the Golgi fraction from uninfected CHO 15B cells could restore transport activity to reactions containing NEM-treated donor and NEM-treated acceptor membranes (Fig. 1*a*). Golgi fractions prepared from uninfected CHO 15B cells possess neither donor activity (as they contain no G protein) nor acceptor activity (as they lack the GlcNAc transferase), and thus do not normally have a stimulatory effect on the assay. However, uninfected CHO 15B Golgi membranes completely restored transport activity to mixtures of NEM-treated donor and NEM-treated acceptor (Fig. 1*a*). This restorative activity is our definition of NSF, which is conveniently assayed because the entire transport process is dependent upon this factor. Presumably, the NSF added in the form of Golgi membranes replaces the endogenous NSF that had been quenched by NEM treatment of donor and acceptor. As expected, pretreatment of the uninfected CHO 15B membranes with NEM abolished their ability to restore transport (Fig. 1*a*).

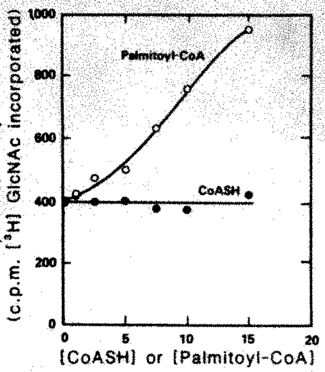

Fig. 3 Effect of palmitoyl-CoA (○) and CoASH (●) on transport at low ATP levels. Final concentration of ATP was 0.5 µM; other conditions were as described in Fig. 1. As the CoASH and acyl-CoA effects are strongest when NSF is present but in limiting amounts, endogenous NSF on the membranes was partially inactivated. (This cannot easily be achieved by adding NSF back to completely NEM-inactivated membranes, because ATP is present in the soluble NSF preparations described.) Partial inactivation was obtained with a relatively mild treatment of donor and acceptor (0.5 mM NEM for 1 min at 0 °C) after which G protein transport is reduced by about 80%, versus the 95% inhibition typically seen using 1 mM NEM for 15 min.

The ability of uninfected CHO 15B membranes to provide NSF suggested that this factor was being released from membranes under the conditions of our transport reaction. To find conditions to extract NSF from the Golgi membranes in a soluble form, high-speed supernatants obtained after various incubations of the CHO 15B Golgi membranes were assayed for their restorative activity (Fig. 1*b*). About 20% of the restorative activity of uninfected CHO 15B Golgi membranes was released into the supernatant after incubation with ATP at 0 °C. Neither the cytosol fraction nor incubation at 37 °C was required for NSF dissociation (Fig. 1*b*). Release of NSF occurred within 5 min at 0 °C and depended on the presence of a nucleotide such as ATP (Fig. 1*b*). Half-maximal release occurred at about 65 µM ATP. ADP, but not AMP, could replace ATP in promoting release (Fig. 1*b*). (Other nucleotides have not been tested.) The significance of the rapid, temperature-independent release of NSF by a nucleotide is not yet clear, but this behaviour suggests that NSF is peripherally bound to the cytoplasmic surfaces of membranes in the Golgi fraction.

This ATP-extracted supernatant was used as the standard preparation of NSF. The soluble form of NSF retained its NEM-sensitivity (1 mM, 0 °C, 15 min) (Fig. 1*b*). The relative molecular mass of NSF is greater than 100,000 as determined by its ultrafiltration characteristics, and it is inactivated by trypsin and by boiling (Table 1). These results imply that NSF is a macromolecule, partially or entirely proteinaceous.

While testing a series of well-known small molecules containing sulphydryl groups as controls to illustrate the specificity of the restorative activity of NSF (which contains an essential sulphydryl), we were struck by the fact that reduced coenzyme A had some restorative activity, although much less than could be obtained with the bona fide (proteinaceous) NSF (Fig. 1*b*). Moreover, CoASH (at 10 µM) greatly stimulated the ability of NSF to restore transport activity to NEM-treated membranes

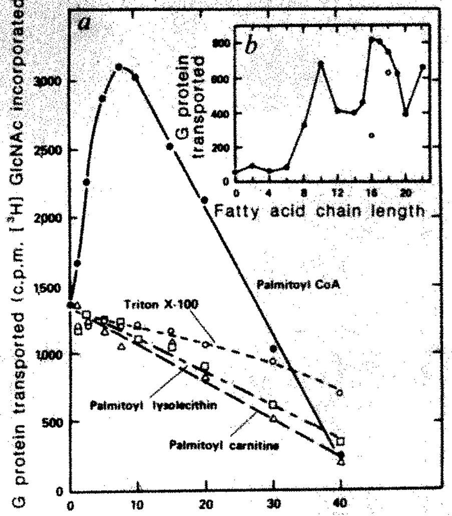

Fig. 4 Comparison of stimulation by acyl-CoA with the effects of detergents. Donor and acceptor were partially inactivated with NEM (0.5 mM for 1 min at 0 °C) as in Fig. 3. *a*, Transport assays were performed as in Fig. 1 (with 50 µM ATP). Palmitoyl-CoA (●), Triton X-100 (○), palmitoyl lysolecithin (△) and palmitoyl carnitine (□) were added as aqueous solutions to the concentrations indicated. *b*, The carbon chain length dependence of stimulatory activity was tested for various saturated fatty acyl-CoAs(●) and the monounsaturated palmitoleoyl ($C_{16:1}$) CoA and oleoyl ($C_{18:1}$) CoAs (○). Chain length 0 corresponds to free CoASH. Fatty acyl-CoAs were added as aqueous solutions to a final concentration of 15 µM. ATP concentration was 0.5 µM. A background of 477 c.p.m. (obtained with no acyl-CoA present) has been subtracted. All reagents were from Sigma.

Table 1 Inactivation of NSF by high temperature and proteolysis

NEM-treated donor and NEM-treated acceptor plus:	[^{3}H] GlcNAc incorporated into G protein (c.p.m.)
A, No addition	283
B, NSF, preincubated 10 min at 37 °C before assay	3,474
C, NSF, boiled 3 min at 100 °C	237
D, NSF, trypsin-treated, then trypsin inhibitor	238
E, NSF, trypsin inhibitor, then trypsin	2,288

Transport assays were performed as in Fig. 1. Concentrated NSF (1.0 mg ml^{-1} protein) was prepared as described in Fig. 2. For samples B and C, 2 µl of water were added to 10 µl of NSF. For sample D, 1 µl of 1 mg ml^{-1} trypsin (Sigma, type XIII) was added to 10 µl of NSF. After 5 min at 37 °C, 1 µl of 2 mg ml^{-1} soybean trypsin inhibitor (Sigma) was added, followed by a further 5 min at 37 °C. For sample E, the order of addition of trypsin and trypsin inhibitor was reversed. Sample B was placed at 37 °C for 10 min to control for the effects of incubation. Five µl of samples B–E were assayed for NSF activity. Sample E was less active than sample B presumably because the inhibition of trypsin was not instantaneous.

Table 2 Dependence of transport of the unacylated VSV New Jersey G protein on NSF and acyl-CoA

	[^{3}H] GlcNAc incorporated into G protein (c.p.m.)
Donor + Accptor	1,473
NEM-Donor + NEM-Acceptor plus:	
A, No addition	184
B, NSF	379
C, Palmitoyl-CoA	237
D, NSF + Palmitoyl-CoA	687
E, NEM-treated NSF + Palmitoyl-CoA	204

Donor membranes were prepared exactly as described[3], except that the infection was done with the New Jersey strain of VSV. Transport assays were performed as in Fig. 1. Immunoprecipitation was carried out using a rabbit antiserum raised against a synthetic peptide corresponding to the carboxy-terminal 15 amino acids of the New Jersey G protein (Thomas Chappell, unpublished). Staph A cells (0.4 ml; Pansorbin, Calbiochem Corp.) were precoated with antibody (0.2 ml serum), and 10 µl of a 20% suspension were added to 50 µl incubations in place of the anti-G protein antiserum we normally use for immunoprecipitation. Where indicated, 1.3 µg concentrated NSF (prepared as described in Fig. 2) and/or 10 µM palmitoyl-CoA were added. For sample E, NSF was treated with 1 mM NEM for 15 min at 0 °C, followed by 2 mM dithiothreitol (DTT).

(Fig. 2*a*). CoASH was not simply acting as a nonspecific reductant, as the CoASH effect was manifest in the presence of excess (0.4 mM) dithiothreitol (Fig. 2*a*) and also because reduced glutathione was totally inactive in replacing CoASH (not shown). Note that the synergy observed with CoASH is greatest with low concentrations of NSF (Fig. 2*a*). Also, neither CoASH nor NSF significantly stimulated control transport reactions containing untreated donor and acceptor membranes, in which saturating amounts of endogenous NSF are present (Fig. 2*b*). These data suggest that CoASH (or some derivative of CoASH) and NSF act together in a step required for protein transport.

The major function of CoA is as a carrier of activated acyl groups, linked by a thioester bond. Desulpho CoA (in which the SH is replaced by H) was inactive (data not shown), suggesting that the CoA effect involves the linkage of CoA through a thioester bond to endogenous acyl groups. If so, then an acyl-CoA, rather than CoASH itself, would be the true cofactor to NSF. As shown in Fig. 2*a*, palmitoyl-CoA replaced CoASH, and offered an even better stimulation of NSF activity. Palmitoyl-CoA did not appreciably stimulate the complete transport reaction in which NSF is not limiting (Fig. 2*b*).

That palmitoyl-CoA was more active than CoASH on a molar basis suggested that this fatty acyl-CoA was acting directly, rather than indirectly by supplying CoASH upon breakdown. The apparent cofactor activity of CoASH would then be the result of the action of endogenous fatty acid-CoA ligase, which catalyses the reaction: endogenous fatty acid + CoASH + ATP $\rightleftarrows$ fatty acyl-CoA + AMP + PP_i. In the absence of this ligase activity, fatty acyl-CoA should still serve as cofactor to NSF, but CoASH should no longer have any cofactor activity. To test this prediction, we took advantage of the fact that fatty acid-CoA ligase (from rat liver microsomes) has a K_m for ATP of about 2–5 mM (refs 10, 11), whereas the transport reaction has an apparent K_m for ATP of only about 1 µM (ref. 9) (although we routinely use 50 µM ATP). Therefore, at very low ATP concentrations, ligase activity should be selectively reduced relative to transport. We found that palmitoyl-CoA retained its cofactor activity but CoASH no longer stimulated NSF in assays containing 0.5 µM ATP (Fig. 3). Presumably, transport at such low ATP levels is normally driven by an endogenous pool of acyl-CoA molecules. These data strongly suggest that fatty acyl-CoA itself acts as cofactor to NSF in promoting transport, although it is formally possible that some other kind of endogenously activated acyl group is enzymatically exchanged for the acyl moiety of acyl-CoA to form the active species. Acyl-CoAs with a broad range of fatty-acid chain lengths had cofactor activity when measured at 0.5 µM ATP to prevent resynthesis (Fig. 4*b*). The activity of short chain derivatives (such as C_8–C_{12}) may be significant, but might also be due to protection of endogenous pools of long-chain acyl-CoA from enzymatic hydrolysis.

What might be the mechanism of action of fatty acyl-CoA in promoting protein transport in the cell-free system? One possibility is that acyl-CoA acts as a bulk detergent[12]. This seems unlikely because although palmitoyl-CoA stimulates NSF-dependent transport, palmitoyl carnitine, palmitoyl lysolecithin and Triton X-100 do not stimulate, but rather inhibit transport, even at the lowest concentrations tested (Fig. 4*a*). Palmitoyl-CoA eventually inhibits the transport reaction (Fig. 4*a*), presumably because of a bulk detergent effect at high concentrations. The stimulation by long-chain acyl-CoAs is strongest at concentrations similar to those found in intact cells[13]. A second possibility is that acyl-CoA acts as an intact molecule in some specific fashion, perhaps as an allosteric effector of an enzyme[14] or as a detergent when locally concentrated by a specific process.

We think the most attractive hypothesis, in line with its general role in cells, is that acyl-CoA acts by donating its fatty-acid chain to a recipient molecule, possibly a protein. Acylation could activate a component of the transport machinery; deacylation would then recycle this component for another round. NSF would be involved in this acylation-deacylation cycle. Energy in the form of ATP would be needed, at least through its use in fatty acyl-CoA synthesis. As G protein is known to be fatty acylated[15], it is conceivable that G protein itself is the acyl acceptor in this postulated cycle. However, the fatty acylation of G protein has been shown to be unncessary for transport *in vivo*[16]. Moreover, G protein is already acylated before it is transported in the cell-free system, and retains its fatty-acid chain during its transport[17]. To rule out directly any involvement of G protein acylation in the stimulation of G protein transport by NSF and acyl-CoA, we prepared donor membranes from CHO 15B cells that had been infected with the New Jersey strain of VSV. Unlike the Indiana strain we normally use, the New Jersey virus contains G protein that is not acylated *in vivo*[18]. As expected, treatment of Golgi membranes with NEM abolished transport of New Jersey G protein, and the restorative activi of NSF was enhanced by palmitoyl-CoA (Table 2). Thus t putative fatty-acid recipient is not the transported protein itse

Finding any small molecule that is needed for a comple cellular process can only help to pinpoint a well-defined, unde lying chemical reaction. Although the exact meaning of t requirement for fatty acyl-CoA in protein transport is not y clear, this result is likely to facilitate a biochemical dissectio

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Stimulation of protein-directed strand exchange by a DNA helicase

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The protein-mediated exchange of strands between a DNA double helix and a homologous DNA single strand involves both synapsis and branch migration, which are two important aspects of any general recombination reaction[1]. Purified DNA-dependent ATPases from *Escherichia coli* (recA protein)[2,3], *Ustilago* (rec 1 protein)[4] and phage T4 (uvsX protein)[5–7] have been shown to drive both synapsis and branch migration *in vitro*. The T4 gene 32 protein is a helix-destabilizing protein that greatly stimulates uvsX-protein-catalysed synapsis[6], and the *E. coli* SSB (single-strand binding) protein stimulates the analogous recA-protein-mediated reaction to a lesser degree[8]. One suspects that several other proteins also play a role in the strand exchange process. For example, a DNA helicase could in principle accelerate branch migration rates by helping to melt the helix at the branch point. The T4 dda protein is a DNA helicase[9,10] that is required to move the T4 replication fork past DNA template-bound proteins *in vitro*[11]. Previously, we have shown that the dda protein binds to a column that contains immobilized T4 uvsX protein[12]. We show here that this helicase specifically stimulates the branch migration reaction that the uvsX protein catalyses as a central part of the genetic recombination process in a T4 bacteriophage-infected cell.

The T4 uvsX protein catalyses branch migration in a directed manner, moving the branch point in the 5′ to 3′ direction with respect to the invading strand[5]. To monitor this reaction, we have employed the assay schematically illustrated in Fig. 1. A 3′ end-labelled double-stranded linear M13 DNA molecule is incubated with a homologous single-stranded DNA circle in the presence of the T4 uvsX and gene 32 proteins and ATP. productive synapsis rapidly ensues at one end of the DN molecule followed by a slower process of branch migration. T measure the extent of branch migration, aliquots are remove at various times and cut by brief exposure to a large excess o *Bam*HI restriction nuclease; the DNA products are then ana lysed by electrophoresis through a denaturing agarose gel[13,1 As *Bam*HI cuts the M13 DNA only once, two radioactive DN bands that are less than full length are expected on autoradiogra phy of the gel (one from each strand), so long as the heteroduple region has not extended beyond the *Bam*HI site. However, a soon as the branch point moves past this site, the displace radioactive strand will no longer be cut, and a full-length stran 6,407 nucleotides (nt) long will appear on the gel, replacing on of the two shorter DNA fragments (Fig. 1).

In the experiment shown in the insert in Fig. 1, the *Bam*H site was located 2,220 base pairs (bp) from the end of the linea DNA molecule at which synapsis occurs, and about thre minutes were required for the branch point to reach this sit As expected, shorter and longer times are required in this assa respectively, if the *Bam*HI site is instead located 474 or 3,54 base pairs from this end (data not shown). We can thereb calculate a rate of branch migration of approximately 15 bp pe second for the uvsX protein-catalysed process. Figure 1 als shows the effect of the T4 dda protein on this reaction. Th addition of this protein was delayed until 1 min, a time that i sufficient to allow complete synapsis (data not shown). Withi 0.5 min after its addition (1.5 min point), a considerable amoun of the full-length DNA strand is observed. Quantitation reveal that the helicase has increased the rate of branch migration t approximately 70 bp s^{-1}, a stimulation of more than 4-fold.

In Fig. 2, autoradiograms of this kind have been scanned wit a densitometer, and the degree of restriction nuclease resistanc plotted as a function of time for experiments in which differen amounts of the dda protein were added. As we have previousl reported for replication fork movement[15], the acceleration o branch migration increases with helicase concentration and al the DNA molecules in the population seem to display equivalen

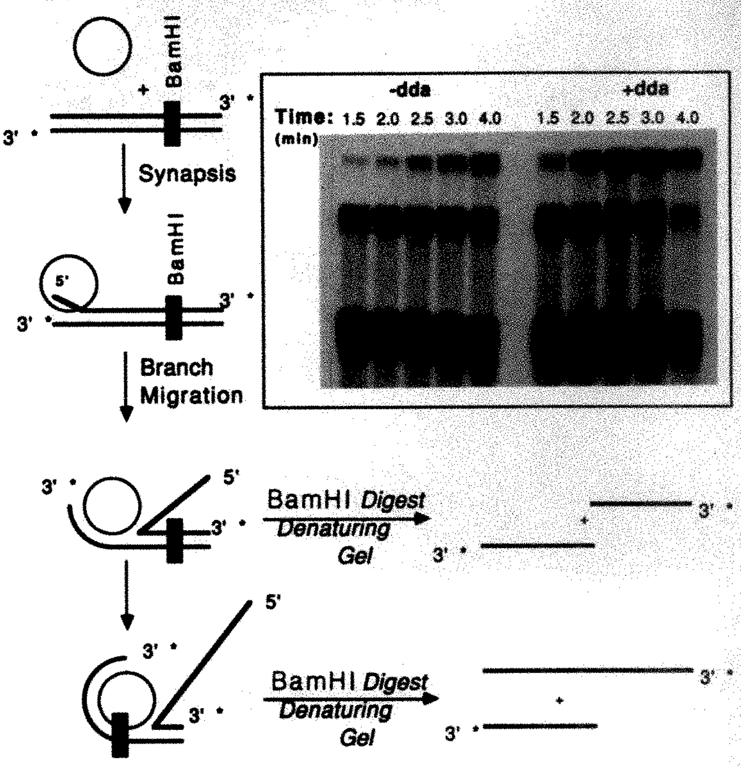

Fig. 1 The rate of uvsX protein-catalysed branch migration is increased by the addition of the T4 dda protein, a DNA helicase. The design of the experiment is illustrated schematically at left and below; inset, autoradiogram showing results. In this experiment, the *Bam*HI site was located 2,220 nucleotides from the end at which synapsis is initiated; therefore, after DNA denaturation, radioactive DNA fragments that are 2,220 and 4,187 nucleotides long should be produced by the *Bam*HI restriction nuclease before the branch point reaches this site, whereas radioactive DNA fragments that are full-length (6,407 nucleotides) and 2,220 nucleotides long should be produced after the branch point passes the site. Although synapsis can begin at either end of the linear double helix, pairing at the opposite end to that shown is unproductive; the directional branch migration that follows will quickly separate the two interacting DNA molecules and prevent strand exchange.
Methods. Each reaction contained 2.5 μM (100 μg ml^{-1}) uvsX protein, 1.5 μM (50 μg ml^{-1}) gene-32 protein, 15.4 μM (as nucleotides) M13 single-stranded DNA, and 6.5 μM 3'-^{32}P-labelled M13 linear double-stranded DNA (produced by treating the replicative form I (RFI) with *Hpa*I, followed by end-labelling with T4 DNA polymerase[22] to a specific activity of 150 c.p.m. pmol^{-1}). The salts present were 10 mM Tris acetate (*p*H 7.4), 10 mM magnesium acetate, 1 mM dithiothreitol, 100 μg ml^{-1} bovine serum albumin (BSA) and 90 mM potassium acetate. An ATP regeneration system (10 mM creatine phosphate and 1 U ml^{-1} creatine phosphokinase) was also present. Each reaction was preincubated at 37 °C for 2 min, and synapsis was then initiated by the addition of 2 mM ATP (time $t = 0$). In the second experiment (+dda), the dda protein was added to a concentration of 0.07 μM (4 μg ml^{-1}) 1 min after the ATP. Aliquots of 10 μl were removed at the times indicated, and mixed with 24 units of *Bam*HI in 10 μl of restriction buffer (10 mM Tris-HCl, *p*H 8.1, 100 μg ml^{-1} bovine BSA serum albumin, 300 mM NaCl, 150 μM poly(dT); the elevated salt in this buffer dissociates the uvsX protein from double-stranded DNA). After 30 s at 37 °C, the reaction was quenched by adding NaOH to 30 mM and EDTA to 20 mM. The samples were then electrophoresed through a 1.2% alkaline agarose gel (30 mM NaOH) at 2 V cm^{-1} for 30 h. The gel was neutralized, dried onto DE-81 paper, and the radioactive DNA visualized by autoradiography. The top, middle and lower bands are 6,407, 4,187 and 2,220 nucleotides long, respectively.

rates. For DNA synthesis, this type of result has been attributed to the distributive mode of action of this DNA helicase, which seems to come on and off the DNA rapidly during the course of an experiment.

Branch migration is likely to be driven by an ATP dependent, 5' to 3' polymerization of uvsX protein monomers along the displaced single strand at the branch point (refs 14 and 16; T.K. and B.M.A., manuscript in preparation). The dda protein functions by first binding to a single strand overhang, and then moving along the DNA in a 5' to 3' direction. It therefore seems reasonable to propose that the dda protein also binds to the displaced strand near the branch point, and then moves rapidly into the helix (Fig. 3). The dda protein could thereby stimulate the growth of the uvsX protein filament, as illustrated. Because of its distributive mode of action, the dda protein presumably falls off the DNA after traversing less than a few hundred base pairs, and the cycle begins again.

Can the dda protein catalyse branch migration by itself? This possibility was tested in an experiment in which an aliquot of a reaction, initiated under our typical conditions, was diluted 10-fold into a solution identical to the original reaction mixture, except that the uvsX protein was omitted. At the resulting 10-fold lower concentration of the uvsX protein, the polar branch migration reaction that it catalyses is abolished (T.K. and B.M.A., in preparation). Adding 4 μg ml^{-1} of the dda protein to the diluted mixture does not affect this result (data not shown). We conclude that the dda-protein-stimulated branch migration illustrated in Fig. 3 requires cooperation between the uvsX and dda proteins, and does not reflect a reaction of the dda protein alone.

Protein affinity chromatography studies have shown that the dda protein binds directly to both the uvsX and gene 32 proteins[12]. Are either of these protein–protein contacts involved in the observed stimulation of branch migration? To explore this question, we first asked whether the dda protein can stimulate the branch migration reaction that is catalysed by the *E. coli* recA protein. The *E coli* recA protein is very similar in its activities to the T4 uvsX protein, and it is known to drive strand exchange with the same polarity as the uvsX protein[3]. Figure 4

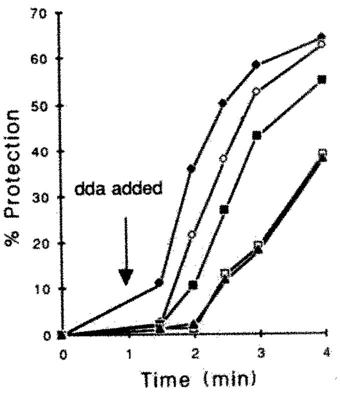

Fig. 2 The rate of branch migration increases with increasing concentration of dda protein. Reactions were conducted exactly as described in Fig. 1, except that the amount of the dda protein added was varied: ◆, 3.8 μg ml^{-1}; ◇, 1.9 μg ml^{-1}; ■, 0.5 μg ml^{-1}; □, none. ▲, Experiment in which the T4 gene 41 protein was added to a final concentration of 0.8 μM (50 μg ml^{-1}) in place of the dda protein and 1 mM GTP was provided. The autoradiograms of the denaturing agarose gels were scanned with a soft laser densitometer, and the percentage of protected molecules was plotted as a function of time.

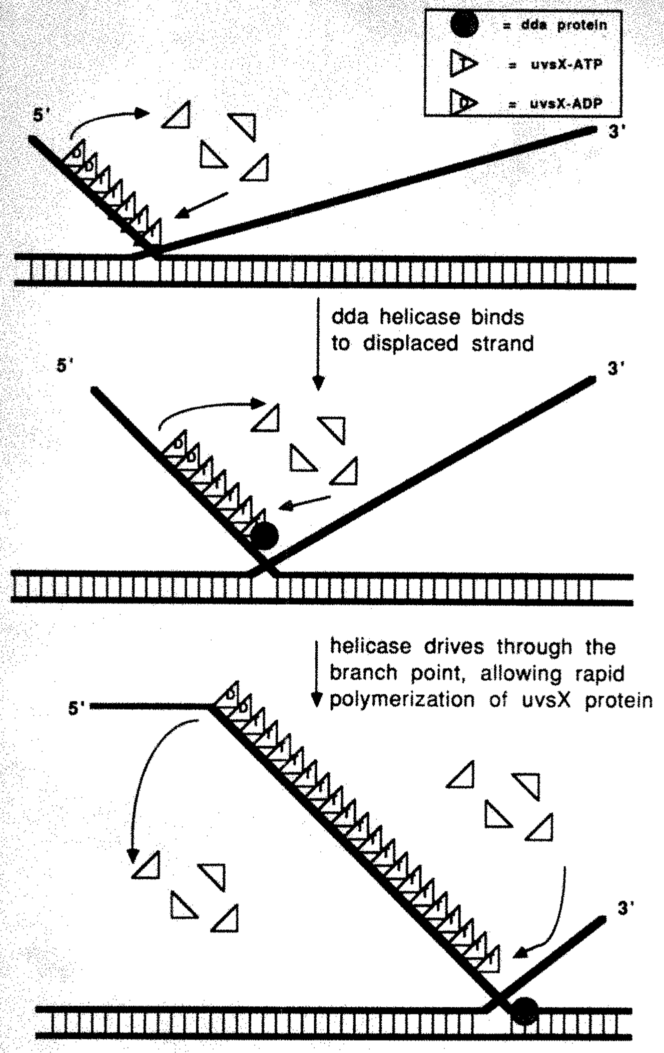
= dda protein
= uvsX-ATP
= uvsX-ADP
5'
3'
dda helicase binds to displaced strand
5'
3'
helicase drives through the branch point, allowing rapid polymerization of uvsX protein
5'
3'

Fig. 3 A model for the stimulation of uvsX protein-driven branch migration by the dda protein (stippled circle). The helicase is loaded onto the displaced strand near the branch point, and moves rapidly into the double helix by translocating itself in a 5' to 3' direction along this strand using the energy of ATP hydrolysis (uvsX-ADP and uvsX-ATP are shown as arrowheads labelled D and T respectively); it thereby facilitates the directional assembly of an uvsX-protein filament and accelerates strand exchange. Efficient action of the dda helicase on the displaced strand may require a direct interaction of the dda protein with the uvsX protein, because the dda protein does not catalyse branch migration efficiently when the uvsX protein concentration is reduced (see text).

shows the result of an experiment similar to the one shown in Fig. 1, except that the *E. coli* recA and SSB proteins were substituted for their T4 analogues; presumably the dda protein does not bind to either of these *E. coli* proteins. In this experiment, the unique *Bam*HI site was located 3,547 bp from the end of the molecule, and significant protection from *Bam*HI nuclease was observed about 20 min after initiation of the reaction. This yields a branch migration rate of 3 bp s^{-1} for the recA-protein-catalysed reaction, in good agreement with the value reported previously by Radding and coworkers[14]. In contrast to the uvsX protein-catalysed reaction, the dda helicase does not accelerate the rate of this reaction (Fig. 4).

Previous work has demonstrated that the T4 gene 41 protein, like the dda gene product, is a DNA helicase that moves along a DNA strand with 5' to 3' polarity[17,18]. However, affinity chromatography suggests that the 41 protein does not directly interact with either the uvsX or gene 32 proteins[12,19]. As shown in Fig. 2, no acceleration of the uvsX-protein-catalysed branch migration rate is detected when the 41 protein is added to the reaction. The same result is obtained when a high concentration of the T4 gene 61 product is added, which stimulates the helicase activity of the 41 protein[18] and completes the T4 primosome[20] (data not shown).

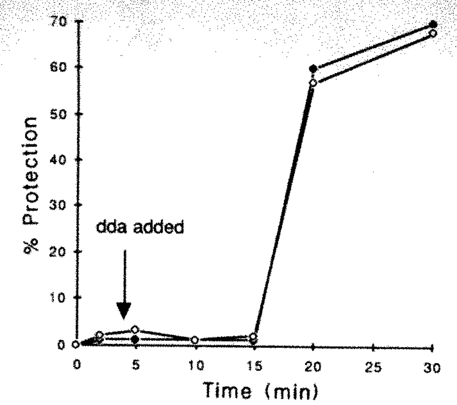

Fig. 4 The dda helicase has no effect on recA-protein-promoted branch migration. The reaction outlined in Fig. 1 was conducted according to Radding and coworkers[14], and the *Bam*HI protection assay was employed to monitor the rate. In this experiment, the double-stranded DNA had been produced by *Bal*I treatment of the RFI M13 DNA, placing the BamHI site 3,547 base pairs from the end at which synapsis begins. In the '+dda' reaction (◆), the dda protein was added at 0.07 μM (4 μg ml^{-1}) 4 min after addition of the ATP (which initiated pairing); ◇, no dda protein added. The autoradiogram of the gel was scanned, and the results plotted as in Fig. 2.

The failure of the dda protein to stimulate the recA-protein-catalysed reaction, and of the gene 41 protein to accelerate the uvsX-protein-catalysed reaction, suggests that specific protein-protein interactions are involved in the observed stimulation of branch migration by the dda protein. As one possibility, this direct interaction could be required for efficient loading of the DNA helicase onto the displaced strand at the head of a uvsX nucleoprotein filament (Fig. 3).

It is becoming increasingly apparent that many complex processes in DNA metabolism, including transcription and replication, are mediated by highly specialized protein complexes that can be viewed as 'protein machines'[21]. The results reported here suggest that general recombination may involve catalytic machinery of comparable elegance.

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Amino-terminal processing of proteins

A. Ben-Bassat and K. Bauer

Recombinant proteins produced in bacteria often retain the initiation methionine at their amino-terminal end. This N-terminal methionine may affect the immunogenicity of the protein, necessitating removal.

PROTEIN synthesis is always initiated with either methionine in eukaryotes or *N*-formylmethionine in prokaryotes and eukaryotic organelles. The *N*-formyl moiety present in the proteins of eukaryotic organelles and bacteria is normally removed by deformylase, leaving methionine at the N-terminus. In the production of many intracellular proteins the methionine is removed by methionine aminopeptidase (MAP) after the initiation of translation. The substrate specificity and universality of MAP among prokaryotes and eukaryotes has been inferred from the N-terminal sequence analysis of mature cytosolic proteins[1,2]. Recently the substrate specificity of MAP was verified using enzyme from *Escherichia coli*.[3]

Influential neighbours

Methionine cleavage from the N-terminal end of the protein is strongly affected by the adjacent amino-acid residue. When the side chain of this penultimate amino acid is large, hydrophobic or positively charged, the methionine is retained; when the side chain is small and uncharged the methionine is cleaved (Table 1). Consequently, the DNA sequence of a gene can be used to predict the fate of the N-terminal methionine of the resulting protein. These conclusions are based in part on qualitative sequence analysis of endogenous cytosolic proteins[1,2]; partial removal of the methionine may be counted as a non-cleavage event. Partial processing of methionine can happen, especially when the penultimate amino acid is Ile, Val, Cys, or another amino acid of intermediate size.

Although no similar studies have been conducted with recombinant proteins, it is reasonable to assume that similar rules apply to their processing. Our experience with recombinant protein expression generally supports the conclusions obtained from endogenous cytosolic proteins. In addition, we found that other factors besides the penultimate residue affect the cleavage of methionine and that the cleavage of the methionine from hyperproduced recombinant proteins is frequently incomplete[3].

Many of the recombinant proteins that are produced for therapeutic uses are based on secretory products from mammalian cells. The N-terminal signal peptides in these secretory precursors are cleaved during the translocation process; thus the mature proteins do not normally contain methionine at the N-terminal end. Examples are γ-interferon (Gln-Asp-), tumour necrosis factor (Val-Arg-Ser-), and human growth hormone (Phe-Pro-). Production of these proteins in *E. coli* intracellularly often results in a product with an extra methionine. Obviously, the biotechnology industry has a strong interest in removing this methionine in order to avoid potential immunogenicity problems, and to obtain a more homogeneous product, without incompletely processed molecules.

Several methods have been used to remove the extra methionine. This first is removal of the methionine by treatment with cyanogen bromide[4]. This method involves chemical modification and requires extra preparation steps. It is not N-terminal specific, and therefore its application is limited to proteins lacking internal methionines.

Table 1 Cleavage of N-terminal methionine in intracellular prokaryotic proteins.

Met cleaved	Met removal varied	Met retained
Ala	Cys	Lys
Gly	His	Arg
Pro	Met	Leu
Ser	Trp	Ile
Thr	Tyr	Asn
	Asp	Phe
	Glu	
	Gln	
	Val	

The data show the effect of the penultimate amino acid on methionine removal[2].

Fusion of the coding sequence of the recombinant protein with a sequence coding for a signal secretory peptide is another method[5]. In this procedure, the fused protein is secreted through the cytoplasmic membrane and cleaved at the fusion site by a signal peptidase. The recombinant protein can then accumulate in the periplasmic space in Gram-negative bacteria or in the growth medium in yeast and Gram-positive bacteria. Unfortunately, secretion of mammalian cell products in prokaryotes and yeast is often limited.

Enzymatic removal of the N-terminal methionine with aminopeptidase has also been used[6]. Here the methionine is removed by incubating the recombinant protein with a peptidase from *Aeromonas proteolytica*. This enzyme is a nonspecific aminopeptidase that cleaves amino acids sequentially from the N-terminal end of proteins. The stop signals for the enzyme are Glu, Asp and x-Pro, where x can be any amino acid except proline[6,7]. This is suitable for removing N-terminal methionine when a stop signal is close to the methionine. The initiation methionine in γ-interferon (Met-Glu-Pro-) and bovine growth hormone (Met-Asp-Glu-) have been removed using this enzyme. When the stop signals are not near methionine, proteolysis by this enzyme can continue, digesting the rest of the protein until it encounters a stop signal. Examples are human growth hormone analogue (Met-Lys-Val-Glu-), where methionine, lysine and valine are removed[6].

The latest advance

The most recent development in N-terminal processing is the *in vivo* and *in vitro* removal of the N-terminal methionine using the MAP enzyme[3]. The MAP gene was recently isolated, cloned and overexpressed in *E. coli* by the authors and their coworkers. The *in vivo* method involves expression of the recombinant protein in a MAP-hyperproducing *E. coli* host. The advantage of this method is that it does not require additional purification steps for both recombinant protein and MAP. The *in vitro* method consists of incubating the purified recombinant protein product with purified MAP. The peptidase can be purified easily from hyperproducing *E. coli* strains, that express MAP at proportions of up to 20 per cent of total cell protein. The utility of these methods was demonstrated by the removal of methionine from interleukin-2 (Met-Ala-Pro-) and ricin A (Met-Ile-Phe-) both *in vivo* and *in vitro*. Recombinant proteins are normally exposed to the native activity of MAP in wild-type cells, but this low concentration often results in incomplete processing. Using an *E. coli* strain with extra MAP-bearing plasmids for the *in vivo* method increases the level of MAP in the cell, assuring more complete cleavage of the N-terminal methionine. In the *in vitro* method, proteins are incubated with a high concentration of enzyme, and the length of incubation can be adjusted to accomplish complete processing. ☐

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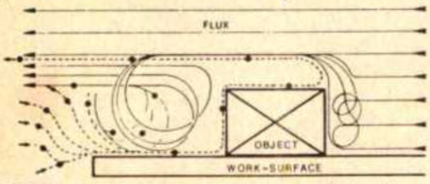
A glimpse of Bio-Expo

Below is a sampling of some of the products to be on exhibit at the Bio-Expo conference held on 24–28 March in Paris, France.

Pharmacia will be showing off its range of products for biotechnology in its booth at Bio-Expo (Reader Service No. 101). Not the least of Pharmacia's offerings will be the CellPhect Transfection Kit, for optimizing high efficiency **transfection of eukaryotic cells** with DNA. The kit contains three solutions; two optimized buffers for calcium phosphate transfection for stable expression, and one DEAE-Dextran solution for transient expression. The kit is supplied with a detailed protocol for both stable and transient expression procedures, and one kit contains enough reagents for 25–100 transfections, depending upon the method used and size of the Petri dish.

With the **sterile storage boxes** for laminar flow hoods developed by ESI-Flufrance, objects needed for sterile procedures may be kept near at hand, without interfering with the laminar flow and introducing the possibility of contamination (Reader Service No. 102). Instead of keeping objects on the work area surface where they can interrupt the air flow, using ESI-Flufrance's side-mounted box allows researchers to keep the work area clear of extra utensils until they are needed. The stainless-steel storage box is equipped with an internal ultraviolet radiation source, so its contents are kept sterile. The box has a glass door that opens directly inside the work area, at an angle designed so that the pivoting axis of the door is always parallel with the direction of the flow, for minimum flow disturbance. ESI-Flufrance has developed the sterile storage boxes for both horizontal and vertical laminar flow hoods. As an option, a second glass door may be added on one of the outer faces of the storage box to convert it into a sterilization chamber.



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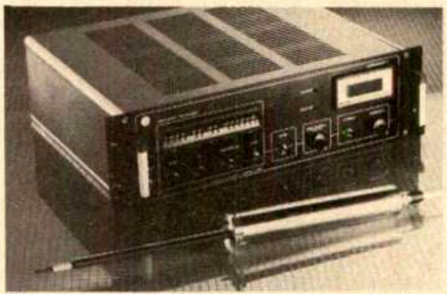
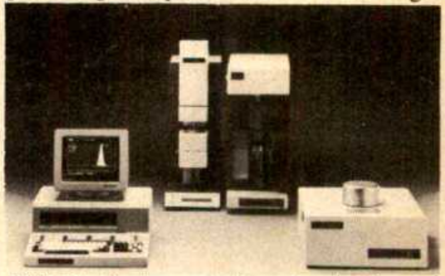
With the **solids probe** for mass spectrometry developed by Kratos Analytical, analyses can be performed under precise temperature control (Reader Service No. 103). The £7,000 (UK) probe's tip is composed of stainless steel and has the heater connected directly to it, allowing rapid heat transfer from the sample, so the recorded and actual temperatures match exactly. According to Kratos Analytical, the solids probe tip temperature can exceed 800°C, and 600°C can be reached from ambient temperature in approximately one minute. The probe is also heatable in atmosphere without damage, and can be cooled, directly to the tip end, with liquid nitrogen or dry air.



The Kratos Analytical probe and control unit.

Hewlett-Packard's new **analytical supplies catalogue** is out, and it now contains information on such products as sample preparation supplies, laboratory robot supplies, filters, vials, flow metres, and even a water purification system (Reader Service No. 104). The free 224-page catalogue also outlines HP's more traditional products: gas and liquid chromatography columns, syringes, chemical samples, and supplies for HP's chromatography and mass spectrometry instruments. The catalogue's appendices, including more than 100 capillary and liquid chromatography column applications, chromatographic equations, miscibility tables, ~~conversion~~ tables, and a glossary of chromatographic terms, make it a handy reference for analytical chemists.

Perkin-Elmer has recently introduced a new line of **thermal analysis** systems, the DELTA series (Reader Service No. 105). Presented as a lower-cost option for laboratories with limited budgets or those who are new to thermal analysis, the £18,500–45,000 (UK) systems are based on the Perkin-Elmer 3700 Data Station as instrument controller. According to Perkin-Elmer this system permits configuration of a low-cost thermal analysis system by providing complete control of a single thermal analyser module. The library of software available for the DELTA series currently includes programs for instrument control, standard and advanced analyses, and multitasking options that allow the DELTA series to perform simultaneous data collection, analysis and plotting.



DELTA: the low-cost alternative for thermal analysis.

Flow is now distributing Gelaire **laminar air flow benches** and Airone **workstations** (Reader Service No. 106). The horizontal laminar air flow benches have automatic air speed control, and the range of five models offers up to 60 options. The benches are available as either table-top or freestanding models, with stainless steel or formica work surfaces. The vertical laminar air flow benches for tissue culture come in three sizes, and also sport automatic air speed control. The Airone fume containment workstations, also built by Gelaire, feature a wide range of activated carbon filters, making them a flexible system for the control of occupational hazards. The Airone range includes modular bench units and a mobile workstation.

Brownlee Labs, a division of Applied Biosystems, Inc., has a new product bulletin available describing the Brownlee SFC System One for **supercritical fluid chromatography** (SFC) (Reader Service No. 107). The free brochure contains a complete description of System One, plus pointers on when to use SFC, and the suitability of different sample types for analysis by SFC. Illustrative chromatograms are also included. □

These notes are compiled by Carol Ezzell from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal and apply only within the country indicated.

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UNIVERSITY OF CAPE TOWN

Posts in Anatomy & Cell Biology

Applications are invited for the posts of Associate Professor/Senior Lecturer and Senior Lecurer/Lecturer in the Department of Anatomy and Cell Biology. Applicants should be well qualified in any of the following areas: neuroanatomy, gross anatomy, histology, or embryology.

Appointment will be made either on a permanent or fixed-period basis, in terms of the Joint Agreement between the University and the Cape Provincial Administration.

Salary ranges: Associate Professor: R32 193-R38 568/R40 194-R48 843; Senior Lecturer: R28 824-R36 018/R37 293-R43 446; Lecturer: R19 044-R30 918/R32 193-R37 293 per annum, with an annual bonus and attractive staff benefits. In addition, a non-pensionable allowance may be payable by the provincial authority under certain circumstances.

Applicants should submit a full curriculum vitae and the names and addresses of three referees, not later than 21 April 1987, to the Registrar (Attention: Appointments Office EH1287), University of Cape Town, Rondebosch, 7700, Republic of South Africa. Further information may be obtained from the above or from the Secretary, SA Universities Office, Chichester House, 278 High Holborn, London WC1V 7HE.

The University's stand against apartheid and all racially discriminating legislation is on record. Information on this as well as on the University's policy not to discriminate in the appointment of staff or the selection of students on the grounds of sex, race or religion is obtainable on request.

(W3913)A

Bates Wells Recruitment CT ● 099R

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an der Abteilung Pathologie der Medizinischen Fakultät der RWTH Aachen ist eine

C3-Professur auf Lebenszeit für Pathologie (Elektronenmikroskopie)

zu besetzen.

Der/die habilitierte Bewerber/in soll Arzt/Ärztin für Pathologie sein und die Allgemeine Pathologie sowie die Spezielle Pathologische Anatomie in Forschung und Lehre vertreten können. Er/Sie soll zugleich die Funktion eines Oberarztes/Oberärztin der Abteilung Pathologie wahrnehmen.

Vom Bewerber/Bewerberin werden eine langjährige Berufserfahrung und umfassende Kenntnisse in modernen Methoden der Morphologie, insbesondere Ultrastrukturforschung, Zellkultur und Immuncytochemie erwartet.

Zu dem Aufgabenbereich gehört die Mitwirkung an der pathologisch-histologischen Diagnostik.

Der/Die Bewerberin wird gebeten, einen Lebenslauf, Darstellung des beruflichen Werdeganges und ein Verzeichnis der wissenschaftlichen Publikationen bis zum 30.4.1987 an den

Dekan der Medizinischen Fakultät der Rheinisch-Westfälischen Technischen Hochschule Aachen, Klinikum, Pauwelsstraße, D-5100 Aachen, zu senden. (W3891)A

Biochemist

Greenford *Salary £10 – £13K*

Glaxo Group Research is Britain's leading pharmaceutical organisation with an outstanding reputation for developing safe and effective new medicines for world-wide markets.

We are now looking for an experienced biochemistry graduate to join our growing Virology Unit. Prime responsibility will be the development of immunoassays and enzyme assays as part of a collaborative project between the Virology and Genetics Units.

You will possess a good honours degree in biochemistry and ideally have worked for the last two or more years in the development of enzyme assays, protein purification and various immunological and immunofluorescent techniques.

In addition to an excellent salary (which includes a Company bonus scheme), other benefits include a non-contributory pension, subsidised canteen and an active sports and social club.

Please send a CV or telephone for an application form to: Ted Smith, Personnel Officer, Glaxo Group Research Limited, Greenford Road, Greenford, Middlesex UB6 0HE. Tel: 01-422 3434, ext. 2934, quoting reference number 20.

(2406)A

Glaxo Group Research Ltd.

BIOCOMPUTING at EMBL

The European Molecular Biology Laboratory is an international research institute located in Heidelberg, West Germany, with major research programmes in Biological Structures, Cell Biology, Differentiation, Gene Structure and Regulation, and in Biocomputing. The Biocomputing programme performs research in theoretical and computational biology. Work is now intensifying in order to meet the challenge provided by the explosive growth in biological sequence and structure data. Major goals are to develop the next generation of biological data and knowledge bases and to better understand the way in which sequence and structure code for biological function. We are therefore looking for several new researchers, research assistants and software specialists in the areas of protein design, evolution of protein structure, molecular dynamics, image analysis, computer graphics, sequence analysis, data base and knowledge base development, and computer system support.

The computing facility at EMBL consists of a VAX cluster (VAX 8600 and VAX 11/785) with terminal servers, SUN-3 work stations, a network of Macintosh personal computers, a PS350 vector graphics display, and data acquisition systems, all connected via a local area Ethernet network. Connections to EARN/BITNET and DATEX-P allows world-wide electronic communication. In 1987 a raster graphics station and a facility for the production of scientific films will be added.

Successful applicants have a unique opportunity to integrate expertise in computer science, mathematics, physics, chemistry, and linguistics into molecular biology - in a truly interdisciplinary effort. In addition to training in one of these areas you should have a strong interest in molecular biology and appropriate programming experience. Prior experience in biocomputing is not a prerequisite.

Apply for one of the specific posts below (Ref. 87/06 to 87/13) or SUBMIT YOUR OWN RESEARCH PROPOSAL (Réf. 87/14).

MOLECULAR GRAPHICS

USER CONSULTANT/SOFTWARE ENGINEER

Réf. 87/06

to work in the computer graphics group. Tasks : develop and maintain molecular graphics software and documentation ; interact with users and guests of the graphics facility; assist with research and teaching projects.

IMAGE ANALYSIS

RESEARCH ASSISTANT/SOFTWARE ENGINEER

Réf. 87/07

to work in the image analysis group on a collaborative project between the biocomputing and biologicaal structures programmes. Tasks : design and implement an image analysis software package, primarily for application to electron microscopy data, with emphasis on a modern user interface and a portable, modular design ; assist with the development of new algorithms, as new experimental methods evolve. Desired experience : image analysis, image formation theory.

BIOLOGICAL AND SYSTEM SOFTWARE

USER CONSULTANT/SYSTEMS PROGRAMMER

Réf. 87/08

to work in the computer group. Tasks : install, maintain and document molecular biology software packages ; consult with EMBL users and guests ; participate in systems maintenance for VAX/VMS and UNIX.

NETWORK ACCESS TO BIOLOGICAL SEQUENCE DATA

SOFTWARE ENGINEER

Réf. 87/09

to work in the data library group. Tasks : develop and maintain network access to the EMBL Data Library by remote users ; maintain and develop existing PC-based software for managing sequence data ; participate in development of new methods for the next generation of biosequence and biomolecular data library.

PROTEIN DESIGN

RESEARCH ASSISTANT/SOFTWARE ENGINEER

Réf. 87/10

to work in the protein structure theory group. Tasks : maintain and develop protein structure and other moleculart biology software ; assist with protein design research.

PROTEIN STRUCTURE KNOWLEDGE BASE

SCIENTIST/POSTDOC

Réf. 87/11

to work with the protein structure theory group in the context of an international collaboration. Tasks : use artificial intelligence methods to develop knowledge base for protein structure modelling and participate in research. Desired experience : LISP or PROLOG.

SEQUENCE ANALYSIS

SCIENTIST/POSTDOC

Réf. 87/12

for research on the analysis of nucleic acid and protein sequences. Tasks : collaborate with experimental molecular biologists on specific projects ; help develop mathematical, information theoretical, artificial intelligence, linguistic or other methods of understanding the biological meaning of genetic sequences and their evolution.

PROTEIN DYNAMICS

SCIENTIST/POSTDOC

Réf. 87/13

for research on the dynamics of protein structure. Tasks : investigate the relation between protein dynamics and protein function ; develop molecular dynamics methods in support fo protein design.

We offer an above-average salary plus family, children's and non-resident allowance, depending on personal circumstances.

Write briefly for an application form, quoting the appropriate
reference to : EMBL, Personnel Section,
Postfach, D - 6900 Heidelberg.

Closing date : 30 April 1987.
Starting date : as soon as possible.

EMBL

(W3932)A

CONTESSE

ISA The institute of animal selection

A world leading company in poultry genetics is adding to its research team by recruiting a

Research scientist

The candidate maximum age 30, will hold a university degree (Grandes Ecoles, PhD or equivalent) in the field of zootechnology and quantitative genetics.
This opening based near Rennes - France offers interesting growth possibilities to a person able to demonstrate scientific discipline combined with acute observation, feeling for animals, and having organisational skills and able to work in a team.
Perfect knowledge of english is indispensable (reading, written and spoken).
All applications will be treated in confidence and should be adressed to the personnel manager - Monsieur GIRARD - Institut de sélection animale - La Bruyère - Le Foeil - 22800 QUINTIN. (W3923)A

MEDIA BA

Universiteit van Amsterdam

The University of Amsterdam, the Department of Chemical Technology seeks within the Faculty of Biology applicants for the position of

professor of applied entomology (m/f)*

Vacancynumber N. 2223.

The new professor will be appointed in the Department of Applied Entomology.
Main areas of interest in applied entomology at the University of Amsterdam are photoperiodism and diapause in insects and mites and ecology of phytophagous mites and their natural enemies.

The candidate is expected:
- to give and lead education in applied entomology
- to conduct and stimulate research in the above mentioned fields
- to perform administrative duties that come with the position.

The candidate should have:
- a broad knowledge of the field of applied entomology and experience in teaching at the university level
- experience with regard to research in population dynamics and population genetics in insects and mites
- experience in administrative duties
- willingness to establish and maintain contacts with national and international institutions and organizations in the field of entomology.

The candidate is expected to have a good command of the Dutch language within two years of the appointment.

Gross salary depends on age and experiences and ranges from Dfl. 6,854.- to Dfl. 9,495.- per month (Dutch Civil Servants Code).

Persons interested should send their curriculum vitae, including a list of publications, quoting vacancynumber - within 3 weeks - to the Chairman appointments Committee, Prof. Dr. K. Verhoef, Bureau Subfaculteit Biologie, Kruislaan 318,
1098 SM Amsterdam, The Netherlands.

* *Women in particular are invited to apply.* (W3918)A

Biotechnolog Research Assistant

A challenging position for protein chemistry special is open at the Rockefel University Biopolymer F cility. Successful applican will have a BS or MS and e perience in peptide or DN synthesis. The facility is pr sently equipped with 2 ga phase sequencers, a peptic synthesizer, and a DNA sy thesizer. Salary negotiabl based on qualifications ar experience. Excellent ben fits and beautiful workin environment. Send resum and letters of recommen ation to: **Personnel Offic Rockefeller University, 123 York Avenue., New Yor N.Y. 10021.** *An Equal Oppo tunity Employer.*

(NW647)A

PhDs, Science Writers Wantec

PhDs and journalists attending scientif and biotechnology meetings in Europ needed to write articles on conferenc developments. Contact John Sterling booth D-13 at BioExpo 87 in Paris durin March 24-28, or write to Genetic En gineering News, 1651 Third Avenue New York, NY 10128.

(NW637)A

UNIVERSITY OF OXFORD

Department of Paediatrics
John Radcliffe Hospital
Headington, Oxford OX3 9DU

An experienced postdoctoral research fellow is required to join a group investigating the genetic basis of bacterial virulence expression. The Department has a strong interest in the biological basis of infectious diseases and the successful applicant must have a research background in molecular genetics and/or biochemistry.

The position is funded by the Medical Research Council until 31 March, 1990, in the first instance. The salary is on Scale RSII (£11,790 - £15,700) currently under review.

Applications in writing with full curriculum vitae including the names and addresses of 3 referees should be sent to: **Professor E R Moxon, Department of Paediatrics, John Radcliffe Hospital, Headington, Oxford OX3 9DU.** Closing date: 23 March 1987. (2379)A

DEPARTMENT OF PHARMACEUTICAL SCIENCES

The Department is now embarking on a major reorganisation of its teaching priorities in response to the Nuffield Report and an expansion of the activities of the Pharmaceutical Sciences Institute, the enterprise which co-ordinates the integrated research work of the Department.

Applications are invited for four vacant positions:

LECTURESHIP IN PHARMACEUTICS (Ref. 879/6)

The person appointed will have a background in pharmaceutical, physical or chemical sciences and will contribute to the undergraduate teaching programme in pharmaceutics. A distinctive feature of the Department's teaching is the integration of pharmaceutics with pharmaceutical chemistry to present an undergraduate syllabus concentrating on drug discovery, drug development and drug delivery. The appointee will be expected to participate in research projects in physical pharmaceutics, drug delivery system design or drug targeting. Applications from pharmaceutical scientists currently working in industry will be particularly welcome.

LECTURESHIP IN MEDICINAL CHEMISTRY (Ref. 8710/6)

The person appointed will have a PhD degree in an appropriate pharmaceutical or chemical discipline and will teach the basic chemistry underpinning the mechanism of drug action and the principles of drug discovery and drug development. Applications from candidates with research experience in peptide chemistry or familiarity with modern developments of computer graphics or high field nmr in pharmaceutical research will be especially welcome. A willingness to collaborate with existing research teams discovering and developing new chemical entities for clinical trial will be a desirable attribute.

LECTURESHIP IN PHARMACY PRACTICE (Ref. 8711/6)

Applicants will be registered pharmacists and teaching responsibilities in pharmacy practice will afford the opportunity of integrating with new practice-oriented teaching initiatives in clinical pharmacy, communication skills development and medical information technology. The successful candidate will join an active group researching into community pharmacy and the expanding role of the pharmacist in health care and will be encouraged to collaborate with scientists in other research groups in the Pharmaceutical Sciences Institute.

TEACHING FELLOWSHIP IN DRUG DEVELOPMENT

(Ref. 8712/6)

The Department seeks to appoint a Teaching Fellow who will assist in the teaching of quantitative techniques employed in drug development and pharmacodynamics to BSc Honours students in Pharmaceutical Sciences; this will occupy approximately 8 hours per week. The Fellow, who should hold a PhD degree in pharmaceutical sciences or chemistry, will be expected to contribute to the basic and applied research efforts of the Cancer Research Campaign Experimental Chemotherapy Group and Drug Development Group in their programmes to bring advanced therapeutic products from the laboratory to clinical trial. The Fellow will conduct research in a new pharmaceutical development laboratory operating to Good Laboratory Practices standards.

Informal enquiries may be directed to Professor M.F.G. Stevens, Department of Pharmaceutical Sciences, telephone 021-359 3611 extension 4170.

*Appointments to **LECTURESHIPS** will be for a period of three years initially with the possibility of renewal or subsequent transfer to a continuing appointment. **Salaries are presently under review.***

*Appointment to **TEACHING FELLOWSHIP** will be for a period of three years with the possibility of renewal for a further period not exceeding three years. **Salaries are presently under review.***

Application forms and further particulars may be obtained from the Personnel Officer (Academic Staff), quoting the appropriate Reference Number, Aston University, Aston Triangle, Birmingham B4 7ET. Tel: 021-359 0870 (24-hour answerphone). Closing date for the receipt of applications is: 24th April, 1987.

(2407)A

ASTON UNIVERSITY

UNIVERSITY OF SOUTHAMPTON

Faculty of Medicine

CHAIR OF CHEMICAL PATHOLOGY AND HUMAN METABOLISM

Applications are invited from scientifically or medically qualified candidates for the Chair of Chemical Pathology and Human Metabolism, which will become vacant in October 1987 following the retirement of Professor B. E. Clayton. Further details may be obtained from the Secretary and Registrar, The University, Southampton, SO9 5NH, to whom applications (11 copies from applicants in the UK) should be sent before 8 May 1987. (2360)A

VISITING INVESTIGATOR

A vacancy exists for an **IMMUNOLOGIST** wishing to spend one or more years in a laboratory investigating the regulation of anti-tumor immunity as a model of the immune response to replicating antigens. Applications from MD's interested in gaining research experience, or established investigators wishing to spend a sabbatical year will be considered. The salary will be $25,000 plus health insurance, and Institute housing is available.

The Trudeau Institute is a private, not-for-profit, basic medical research institution located on the shore of Saranac Lake, and near Lake Placid, NY.

Send curriculum vitae and names of three references to: **Dr Robert J. North, Director, Trudeau Institute, Inc., P.O. Box 59, Saranac Lake, NY 12983.** (NW579)A

FACULTY POSITIONS IN RADIOLOGICAL SCIENCES

The Department of Nuclear Engineering at MIT requests applications for **two** positions in the area of radiological sciences. The positions will be on a tenure track with a 3-year appointment as assistant or associate professor to be determined by the qualifications of the successful candidates. These successful candidates will be members of the Radiation Science and Technology program of the Nuclear Engineering Department and may also have adjunct appointments in the Whitaker College of Health Sciences, Technology and Management depending on their interests and qualifications. Candidates should hold a PhD or ScD in an engineering or physical sciences specialty and should have experience in radiation production, detection, dosimetry and/or application. Candidates for one of the positions should have a broad interest in the physics and engineering of radiation therapy including systems for radiation delivery, dosimetry, treatment planning and related computer applications. Candidates for the other position should be interested in a broad range of topics dealing with radiation biophysics including studies of radiation effects and the use of radiation modifying agents.

Independent of the area of research, the candidates should be capable of mounting an extensive research program and obtaining support. The program should interact with and expand other activities in the Nuclear Engineering Department involving nuclear reactions. The candidates should be capable of teaching a wide range of courses in the academic areas of radiation science and technology including radiation and radioisotope applications and experimental nuclear physics and should be able to assist in the radiation health physics curriculum. The teaching activities will form an integral part of the radiological sciences program and the successful candidates will have responsibilities for the supervision of student research including master's and doctoral research.

Candidates for both positions are strongly encouraged to include utilization of the MIT Research Reactor in the formulation of their proposed teaching and research activities. Both candidates should also be able to integrate their research and teaching with other aspects of the department's radiological sciences program such as biomedical imaging and diagnosis. Effective collaboration with other relevant groups in the Boston area is important.

Please submit curriculum vitae to:

Professor Gordon L. Brownell, Massachusetts Institute of Technology, Whitaker College of Health Sciences, Technology and Management, Room E25-330B, Cambridge, Massachusetts 02139.

MIT is an Equal Opportunity/Affirmative Action Employer.

(NW638)A

UNIVERSITY OF ZURICH

(Philosophische Fakultät II)

The Institute of Plant Biology invites applications for an

ASSISTANT PROFESSORSHIP IN PLANT MOLECULAR BIOLOGY

An active interest in host-parasite systems is desirable but not necessarily required. The position includes teaching at the undergraduate and graduate level. The successful candidate is expected to acquire proficiency in German within two years.

Applications, with curriculum vitae, record of teaching and research activities, and a list of publications (no publications, please) should be addressed to:

Prof. Ph. Matile
Institute of Plant Biology
University of Zurich
Zollikerstr. 107
CH-8008 Zurich, Switzerland

(W3914)A

CRUSTACEAN AQUACULTURE

A temporary marine scientist research position is anticipated beginning 1 June 1987 for a PhD (experienced MS considered) to join established shrimp aquaculture research group. Expertise in reproduction, genetics or culture of penaeid shrimp is required. Funding is anticipated for at least 12 months, with excellent potential for multi-year grant support. A starting salary of $25,000 is offered. Interested applicants should submit a curriculum vitae and the names and addresses of 3 references by 15 April 1987 to: Dr Paul A. Sandifer, Director, South Carolina Marine Resources Center, PO Box 12559, Charleston, SC 29412. Tel: (803) 795-6350.

An Equal Opportunity Employer (NW639)A

POSTDOCTORAL POSITIONS ENZYMOLOGY AND PROTEIN CHEMISTRY

Positions available immediately for individuals with Ph.D.'s in biochemistry, molecular biology or related areas to study two control enzymes of glucose metabolism.

Phosphorylase Kinase (Skeletal Muscle) — Subunit interactions, recognition sites, architecture, reconstitution, structures and roles.

Phosphoenolpyruvate Carboxykinase (Liver) — Active site mapping (GTP and divalent metal binding sites and identification of critical Cys) and catalytic fragments.

Newly remodeled, fully equipped laboratories and competitive salaries. Please send curriculum vitae and names and telephone numbers of three references to: Dr. Gerald M. Carlson, Department of Biochemistry, University of Tennessee, Memphis, The Health Science Center, 800 Madison Avenue, Memphis, TN 38163.

The University of Tennessee is an Equal Opportunity Employer

(NW568)A

UNIVERSITY OF SASKATCHEWAN

COLLEGE OF PHARMACY

MASS SPECTROSCOPIST

A mass spectroscopist is required to work with the Drug Metabolism-Drug Disposition Research Group in the College of Pharmacy, University of Saskatchewan. The position involves the operation and routine daily maintenance of VG Micromass MM16 and 7070HE mass spectrometers and associated data systems. Familiarity with modern techniques such as positive and negative chemical ionization, HPLC/MS and fast atom bombardment, would be advantageous. Salary in accordance with experience. Applications including curriculum vitae and the names of three references, should be sent to: **Dr K.K. Midha, College of Pharmacy, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 0W0, Canada (306) 966-6370.**

(NW652)A

MEDICAL RESEARCH COUNCIL

National Institute for Medical Research

Laboratory of Embryogenesis

This is a three-year postdoctoral appointment to work on sequence specific DNA binding proteins that regulate genes active in early development and which are involved in establishing the vertebrate body plan. We believe this is an exciting opportunity for a biochemist/molecular biologist interested in devising technology that will lead to new kinds of embryological investigation. The successful candidate would be involved in further developments of an affinity competition technique already established at NIMR, for which there are many potential applications and ample opportunity to collaborate with other groups within the Institute. (Ref: EG/871, closing date Friday 10th April).

Physical Biochemistry Division

A vacancy has recently arisen for a post of Postdoctoral Scientist to work on a project using high resolution nuclear magnetic resonance (NMR) spectroscopy) to investigate the specificity of nucleotides binding to proteins. Excellent facilities for NMR (Bruker AM500, AM400 and WM200 spectrometers) and molecular graphics (IRIS 3020) are available for these protein-ligand binding studies in the Biomedical NMR Centre at the Institute.

Candidates should have experience of modern NMR techniques (such as two dimensional NMR methods) and preferably have post-graduate experience in an NMR laboratory working on biological problems. Those with experience in protein isolation and purification or in the use of molecular graphics are particularly urged to apply but all applicants with biological NMR experience will be considered.

Further details about this post can be obtained by contacting Dr J. Feeney at the Physical Biochemistry Division. (Ref: PB/872, closing date Friday 1st May).

Developmental Biochemistry Division

Applications are invited from postdoctoral candidates for an appointment, initially for three years, to work on problems concerning the structure and function of genes during development and on their regulation by hormones and growth controlling agents. The project will deal with genes coding for egg coat and yolk proteins and the role of steroid hormone receptors and tissue-specific transcription factors in their regulation. The successful candidate will join a small group working with Dr J.R. Tata, FRS, and there will be ample opportunity for collaboration with other groups interested in similar areas of molecular, cell and developmental biology. The work will involve extensive exploitation of the latest techniques of recombinant DNA, gene transfection and tissue culture, so that experience in these techniques will be an important consideration. Ref: DBM/873, closing date Friday 10th April).

Salaries for all positions will be in the range £9,495—£15,700 plus £1,393 London Allowance per annum. There is also superannuation provision and a substantial pay award pending.

Would all interested please apply (quoting the appropriate reference number) to Mr C.R. Russell, Head of Administration, NIMR, The Ridgeway, Mill Hill, London, NW7 1AA enclosing full curriculum vitae, with the names of two referees and outlining research experience and interests.

Please note the separate closing dates. *The Council is an Equal Opportunities Employer.* (2392)A

IDEC Incorporated

IDEC Incorporated, a recently formed biotechnology company located in Mountain View and La Jolla, California is a leader in research involving immuno-regulation with anti-idiotype antibodies. IDEC is currently conducting clinical trials at leading institutions with its proprietary technology. IDEC currently has excellent career opportunities for the following positions:

Research Director: Experienced research manager sought to lead corporate research programs. Candidate must have a recent record in the conception, execution, and management of innovative biological research, preferably in a product oriented environment. Candidate must demonstrate the ability to contribute meaningfully to the corporate research and development planning process, and to successfully lead scientists in pioneering biological research. Suitable candidate should possess a PhD and/or MD or equivalent degree specializing in cellular biology and/or immunology with several years of managing experience.

Senior Scientists/Research Scientists: All levels of scientists with experience in immunoregulation, anti-idiotype network immunobiology, and molecular immunology are sought. Successful candidates should have academic or industrial experience with the ability to lead research programs. Suitable candidates must possess a PhD, and/or MD or equivalent degree.

Research Assistants: Candidates should have at least two years experience in a immunology lab. Experience should include technical expertise in hybridoma anti-body development, tissue culture, immunochemistry and related areas. Candidate must have a BA/BS or MS.

For prompt, fully confidential consideration, please send your resume to **Personnel Director, IDEC Incorporated, 291 North Bernardo Avenue., Mountain View, Ca. 94043.** (NW653)A

Executive Director of Publications

Due to the tragic death in an automobile accident of Steve Prentis, Executive Director of Publications and founder and Executive Editor of the new journal *Genes & Development*, **Cold Spring Harbor Laboratory** seeks to fill this demanding position immediately. Responsibilities include the energetic continuation of this primary journal and the active development and acquisition of new publications projects.

The ideal candidate should have a broad knowledge of molecular biology, wide-ranging scientific contacts, diplomatic skills, and a strong sense of commitment to the highest level of scientific publishing. Gold Spring Harbor Laboratory offers an ideal working environment, excellent benefits, and a salary commensurate with experience. Please send CV to:

James D. Watson, Director
Cold Spring Harbor Laboratory
Box 100
Cold Spring Harbor, N.Y. 11724

(NW649)A

CSH **Cold Spring Harbor Laboratory**

An equal opportunity employer

Join An Emerging Leader in

DNA PROBE TECHNOLOGY

The GIBCO/BRL Division of Life Technologies, Inc. is expanding its program to develop important novel approaches for isolation and non-radioactive detection of specific nucleic acid sequences.

We are seeking:

SENIOR BIOCHEMISTS or MOLECULAR BIOLOGISTS, Ph.D. and/or M.S.

- Wide range of expertise in basic techniques of molecular biology molecular immunology.
- Proven record of accomplishments (including publications) and demonstrated ability to work independently and creatively.
- Experience with viral sequences of clinical relevance is desirable.

We are a successful biotechnology company with a strong reputation in the industry. The leadership enjoyed by our GIBCO and BRL products in molecular and cell biology is evidence of our commitment to excellence.

Our salary and benefits program is highly competitive. Our location combines the scenic countryside of Maryland with the cultural vitality of Washington, D.C.

Candidates should send resumes to:

Mr. Richard L. Hotaling, Jr.
GIBCO/BRL Division, Life Technologies, Inc.
8717 Grovemont Circle, Gaithersburg, MD 20877

GIBCO/BRL (NW650)A

An equal opportunity employer, M/F/H/V

Faculty Openings Biotechnology

Faculty Positions are available at the Full Professor, Associate Professor and Assistant Professor levels in The Center for Biotechnology at Baylor College of Medicine. This Center is located in a new Baylor building in The Research Forest of The Woodlands, a 25,000 acre wooded community 30 miles north of Houston. The Center consists of the following divisions: (I) Hybridoma Technology, (II) Recombinant DNA Technology, (III) Protein Engineering, (IV) Bio-organic chemistry, (V) Vision Research and (VI) Cellular and Molecular Neurobiology. Applicants should have research backgrounds in one of these areas. Exceptional candidates may also be considered for positions as Division Heads. Although academic excellence is the determining factor in the selection process, experience and interest in biotechnological applications are also desirable.

Please send resume, names of several references and a brief description of research experience and future goals to:

Dr. Dominic Man-Kit Lam,
Director
The Center For Biotechnology
Baylor College of Medicine
4000 Research Forest Drive
The Woodlands, Texas 77381

(NW658)A

An Equal Opportunity A/A Employer

APPOINTMENT OF

DIRECTOR

AT THE ROYAL HORTICULTURAL SOCIETY'S GARDEN WISLEY, WOKING, SURREY

Applications are invited for the post of Director for the RHS Garden, Wisley, Surrey.

The successful applicant is likely to possess a degree or degree equivalent in Horticulture/Botany, or a relevant equivalent qualification combined with proven administrative and managerial ability and experience.

Further details of the post may be obtained by applying in writing to: **The Director General, The RHS Garden, Wisley, Woking, Surrey, GU23 6QB,** or telephoning Guildford (0483) 224234. Completed applications must be received not later than April 6 1987. (2386)A

POSTDOCTORAL POSITION

HEPATIC MEMBRANE TRANSPORT

Postdoctoral position available for NIH-funded project involving characterization and isolation of hepatic membrane proteins mediating bile acid and calcium transport. Applicant must have recent PhD and previous experience in vesicle transport or protein purification techniques. Send curriculum vitae and names of three references to: **Bennett L. Blitzer, M.D., Liver Study Unit, University of Cincinnati College of Medicine, 231 Bethesda Avenue (Room 6560), Cincinnati, OH 45267.** (NW641)A

THE BIOCHEMICAL SOCIETY

requires an

Editorial Assistant

for its Editorial Office in central London. The successful candidate will be required to carry out copy-editing, proof-reading and related work on the Society's journals and books, in particular the *Biochemical Journal.*

Applicants should have a degree (preferably a higher degree) in biochemistry or a related subject, a good command of the English language, and the ability to communicate with authors and editors. Scientific publishing experience would be a considerable advantage. Starting salary £8,000 to £11,000; the exact level will be determined by the successful candidate's experience.

Applications, with full curriculum vitae and the names of two referees, should be sent by 31 March 1987 to the **Editorial ager, Biochemical Society, 7 Warwick Court, High Holborn, don WC1R 5DP.** (2383)A

DIRECTOR FOR THE GRAY LABORATORY

The Cancer Research Campaign invites applications for the Directorship of its Gray Laboratory, Mount Vernon Hospital, Northwood, which will become vacant on 1 April 1988 on the retirement of Professor Jack Fowler.

The work of the Laboratory is concerned with basic and applied aspects of radiobiology in relation to cancer therapy.

The appointment will be made on the Professorial scale. Applications (three copies) with the names of three referees should be submitted to **The Director, Scientific Department, Cancer Research Campaign, 2 Carlton House Terrace, London SW1Y 5AR** from whom further particulars should first be obtained.

The closing date for receipt of applications is 15 May 1987.

Cancer Research Campaign

(2413)A

A highly qualified Toxicology Center in Rome

is seeking an

experienced scientist

endowed with great organizing qualities to fill a challenging top management position.

The succesful candidate will be responsible for organizing the activities of the Center and for promoting its high scientific image. He will be assisted, whenever necessary by internationally recognized experts.

An attractive salary — relative to qualifications and experience — and cultural opportunities at the highest level are offered.

Qualified candidates are kindly requested to submit their curriculum vitae to:

A. MENARINI S.A.S — Pharmaceutical Industries —
Via Sette Santi, 3
50131 Florence, Italy.

Correspondence will be kept strictly confidential. (W3919)A

MRC CLINICAL RESEARCH CENTRE (Northwick Park Hospital)

Watford Road, Harrow
Middlesex, HA1 3UJ

IMMUNOHISTOCHEMIST/ CELL BIOLOGIST

Applications are invited from Research Scientists to work on a project funded by the Cancer Research Campaign on the immunohistochemistry of embryonic and tumour-associated antigens recognised by monoclonal antibodies. The successful candidate, who must have a PhD or equivalent advanced research training will join the applied immunohistochemistry research group headed by Dr Ten Feizi.

Previous experience in immunochemical or immunohistochemical techniques and cell micromanipulations will be an advantage. The position is funded for 18 months in the first instance, with a good possibility of renewal for a further three years. Salary is dependant on qualifications and experience, and will be at an appropriate point on the scales for either university non-clinical academic staff, or NHS medical laboratory scientific officer.

Application forms and further details available from Personnel Section 01 864-5311 ext. 2673 quoting ref. no. 108/1/CRC.

Closing date for completed applications 3rd April, 1987.

The Medical Research Council is an Equal Opportunity Employer. (2398)A

SENIOR AND JUNIOR STAFF AND POST-DOCTORAL POSITIONS

in Molecular Biology, Clinical Research, Haematology, Immunology, Cell Biology and Protein / Peptide Chemistry.

The scientific objectives of The Biomedical Research Centre are:

- to provide an internationally recognized centre of excellence in Western Canada for experimental medicine and related biological sciences;
- to bridge the gap between clinical sciences and the laboratory to expedite the development of new therapeutic agents.

The Biomedical Research Centre is a joint venture between The Terry Fox Medical Research Foundation, a British Columbia Charitable Foundation, and The Wellcome Foundation Limited, of the U.K. In late 1987 The Biomedical Research Centre will move into new laboratories now under construction adjacent to The UBC Health Sciences Centre Hospital, on the campus of The University of British Columbia. The building has been designed and equipped for molecular biology, immunology, cell biology, protein chemistry and peptide synthesis. Research will initially centre upon polypeptide regulators of cellular division and differentiation and will include analysis of structure-function relationships, biology and physiology and roles in inflammatory, allergic and neoplastic diseases.

Staff will be eligible for cross-appointments in appropriate departments in the Faculties of Medicine or Science at The University of British Columbia. Staff with the appropriate medical qualifications may hold clinical appointments within the Hospital.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada.

Applications should include a full C.V. and bibliography, the names of three referees and a short summary of future research interests. Applications or enquiries should be addressed to **The Director, Professor John W. Schrader, at the address below.**

The Biomedical Research Centre
The University of British Columbia
Vancouver, B.C. V6T 1W5

(NW643)A

THE UNIVERSITY OF ARIZONA

DEPARTMENTS OF BIOCHEMISTRY
AND THE ARIZONA CANCER CENTER

FACULTY POSITION

BIOCHEMISTRY/CANCER BIOLOGY

Applications are invited for a tenure track faculty position in the field of Biochemistry as broadly related to cancer, to augment our existing faculty group. Research in the areas of regulation of proliferation and differentiation, lymphokines, growth and regulatory factors, cell-cell interactions, biochemical pharmacology, and the biology of tumor cells is of special interest. However, outstanding scientists in other areas will also be considered.

Candidates for this position should have at least two years of pertinent postdoctoral experience. The successful candidate will be expected to develop a nationally recognized independent research program. A wide variety of interactions with our present faculty members is possible in many areas of biochemistry, immunology, pharmacology and molecular & cellular biology. We provide excellent physical facilities, a stimulating research environment, and competitive salary and benefits. The newly completed Arizona Cancer Center facility and a newly formed Biotechnology Division add to the many scientific resources available at the University of Arizona. Applicants should provide a curriculum vitae, two copies of recent representative publications, and a detailed description of research goals. The applicant should also arrange for three letters of recommendation to be sent directly to us. Closing date for applications April 15, 1987.

The University of Arizona is an Equal Opportunity/Affirmative Action Employer and applications from female and minority group members are strongly encouraged.

Submit applications to: **Jeffrey M. Trent, PhD, Head Biochemistry/Cancer Center Search Committee, Arizona Cancer Center, Room 3945, The University of Arizona, Tucson, AZ 85724.**

(NW644)A

Sandoz Ltd., a leading Swiss-based International company with a strong commitment to pharmaceutical research, is offering a

postdoctoral position

in their newly created Biotechnology department. The successful candidate will be an ambitious Ph.D. in molecular biology with interest in the regulation of eukaryotic transcription.

The position is available immediately for a period of two years.

Applications with curriculum vitae should be sent to:

Sandoz Ltd., Personnel Department, Ref. 7801,
attn.: Mr. R. Zbinden, P.B.,
CH-4002 Basle/Switzerland

SANDOZ

(W3917)A

THE UNIVERSITY OF AUCKLAND

New Zealand

LECTURESHIP IN THEORETICAL & APPLIED MECHANICS

(School of Engineering)

Applicants should be suitably qualified with academic and/or professional experience in one or more of the areas that fall within the scope of the Department's activities. The interests of the Department are mainly in the mathematical modelling and computational solution of engineering problems but also in applied mathematics. At present the two major fields of interest are Continuum Mechanics and Operations Research, but applications in other areas will be welcome. For this appointment the Department would particularly welcome applications from persons with experience or interests in professional engineering.

Commencing salary will be within the range for Lecturers NZ$30,500—$35,000 per annum. An exceptionally well qualified candidate may be appointed at Senior Lecturer level, salary maximum NZ$47,000. Conditions of Appointment and Method of Application are available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF; or the Assistant Registrar (Academic Appointments), University of Auckland, Private Bag, Auckland, New Zealand, to whom applications, in accordance with the Method of Application, should be forwarded as soon as possible but no later than the closing date 21 May, 1987. (W3930)A

CHARING CROSS AND WESTMINSTER MEDICAL SCHOOL

(University of London)

Applications are invited from suitably qualified graduates for the post of Research Assistant for biochemical studies relating to human and experimental diabetes. The appointment will be for at least two years and will be based at the Westminster Children's Hospital, Vincent Square, London, SW1. Salary according to Research Assistant Scale 1A in the range of £8,020—£10,375 (under review) per annum plus London Allowance of £1,393.

For further information contact Dr Bhatt, telephone 01-828-9811, ext 392. Applications with full curriculum vitae and the names and addresses of two referees to The Secretary, Charing Cross and Westminster Medical School, The Reynolds Building, St Dunstan's Road, London W6 8RP, to be submitted within two weeks of the appearance of this advertisement. (2371)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL

(University of London)

POSTDOCTORAL RESEARCH FELLOW

A Biochemist/Molecular Biologi is required to join a departme involved in the application of bi technology to the diagnosis a alleviation of human disease. Th project is to study the mechanism of protein secretion with the aim improving the expression of ther peutically useful proteins. Th work will involve the use coupled *in vitro* transcriptio translation of secretory protei from manipulated DNA seque ces in the presence of intracellula membranes from pancreas.

The project is funded jointly b the MRC and Amersham Inte national for 3 years. The successfu candidate will be able to make us of the facilities and expertise avai able in the Molecular Biolog Department at Amersham. would suit a candidate with exper ence in recombinant DNA techniques, protein translation *in vit* or isolation of intracellular mem branes plus familiarity *wit* modern techniques of biochemica analysis.

Starting salary is £9,000 with a increase of 16% pending plus London allowance of £1,393 pe annum. Applicants with, or expecting to obtain, a PhD shoul send their curriculum vitae wit names and addresses of two referees to Dr Brian Austen, Peptide Unit, Department of Surgery St George's Hospital Medica School, Cranmer Terrace, London SW17 0RE (01-672-1255 ex 5150). Quote reference numbe 41/87. (2377)A

UNIVERSITY OF OXFORD

Dyson Perrins Laboratory

POSTDOCTORAL RESEARCH POSITION

Applications are invited for Cancer Research Campaign funded post to develop a novel class o bis-intercalating drugs as potentia antitumour and antibacteria agents as well as for investigatin the tertiary structure of supercoiled DNA. The work will b directed by Dr G Lowe and Dr P Cook. Experience in syntheti organic chemistry is essential.

The appointment will be for on year in the first instance, with th possibility of renewal, starting on July or as soon as possible thereafter. Salary will be on the RA1 scale which is under review, th starting point depending on ag and experience.

Applications, with curriculu vitae and the names of two referees, should be sent to Dr Lowe, FRS, Dyson Perrins Laboratory, South Parks Road Oxford, OX1 3QY. (2368)A

UNIVERSITY OF LONDON

READERSHIP IN DENTAL ANATOMY AT KING'S COLLEGE LONDON (KQC)

e Senate invite applications for
e above Readership tenable at
ng's College London (KQC) in
e Department of Anatomy and
uman Biology. It is hoped that
e successful applicant will take
duties not later than 1 October
87.

Applicants, who must have
perience in teaching Special
ental Anatomy, should be in-
lved in and plan to continue
rsonal involvement in an active
search programme. This res-
arch programme need not be
rectly concerned with teeth and
eir supporting structures: appli-
ants with wider research interests
specially in Cell Biology, Dev-
opmental Biology and Neuro-
ology) will also be seriously
onsidered.

Applications (10 copies) should
e submitted to the Teachers'
ection (N), University of
ondon, Senate House, Malet
treet, London WC1E 7HU, from
hom further particulars should
rst be obtained.

The closing date for receipt of
pplications is 24 April 1987.

(2362)A

UNITED MEDICAL AND DENTAL SCHOOLS

INSTITUTE OF DERMATOLOGY

(St. Thomas's Campus)

DEPARTMENT OF CELL PATHOLOGY

POSTDOCTORAL RESEARCH ASSISTANT

The project involves the development of immunoelectron microscopical techniques for the localization of basement membrane antigens in fetal and post-natal skin. The work is highly relevant to diagnostic studies and basic research into genetic and acquired blistering disorders. Previous experience in EM and immunocytochemical techniques is desirable.

The appointment is for one year in the first instance with the possibility of extension for a further two years. Salary scale in Range 1A.

Written applications (no form provided) should include c.v. with names of two referees and be sent to Dr R.A.J. Eady, Institute of Dermatology, Dept. of Cell Pathology, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH. For further information contact Dr Eady (tel: 01-928 9292, ext: 3318).

(2380)A

THE UNIVERSITY OF SYDNEY

RESEARCH APPOINTMENT IN DEVELOPMENTAL NEUROBIOLOGY

Reference No: 9/17

Neurobiology Research Centre

Research projects in the research centre are aimed at elucidating the mechanisms which govern the formation and elimination of synapses during development and regeneration of the nervous system. Applications are now invited for the position of Senior Protein Biochemist at the Research Fellow or Postdoctoral level. Initial appointment for one year with prospects of renewal. The successful applicant will be required to play a leading role in a project which is aimed at the isolation of trophic material for central neurones. Applicants should therefore have expertise in aspects of protein separation and purification.

Salary range: Research Fellow $A27,859 - $A36,600 per annum, Postdoctoral Fellow $A24,013 - $A26,808 per annum.

Applications, quoting reference no, including curriculum vitae, bibliography (and three selected reprints) and the names and addresses of two referees, to the Registrar, University of Sydney, NSW 2006, Australia, by 3 April 1987, or as soon as possible thereafter.

(W3921)A

BREWING RESEARCH FOUNDATION

PLANT PHYSIOLOGIST/ ELECTRON MICROSCOPIST

We have a vacancy for a scientist to study the structure of barley grain in relation to malting and brewing. Applicants should have a good honours degree in Botany, Plant Science or a related subject and experience in electron microscopy, preferably with botanical subjects. A postgraduate degree or industrial experience related to cereals would be an advantage.

Initial salary, within the range £8,328 - £10,366 per annum is dependant upon qualifications and experience.

The Foundation is the national research organisation of the UK Brewing Industry, and is situated in pleasant surroundings near Redhill, 30 miles south of London.

Applications, including full curriculum vitae and the name and addresses of two referees, should be sent to: Mr Alan Clapp, The Assistant Director, Brewing Research Foundation, Lyttel Hall, Nutfield, Redhill, Surrey RH1 4HY, quoting Ref: MW/PP.

(2385)A

Pharmacologist

Gastrointestinal Research

Harlow, Essex

Beecham Pharmaceuticals, one of the world's largest health care companies, is committed to a major expansion in research. As a result of this, we now have an excellent opportunity for a Pharmacologist or Physiologist to join our research team investigating gastrointestinal diseases.

Applicants should have a general interest in gut motility, a desire to understand the mechanisms of gut disease and be experienced in such techniques that are used in the measurement of gut motility, propulsion, myoelectric activity and reflex activity in conscious animals.

If you have a relevant post graduate qualification (possibly with post doctoral experience) and the drive and enthusiasm to contribute to our research team we would be interested to hear from you.

We offer excellent conditions of employment including competitive salary, non-contributory pension and flexible working hours. We shall assist with relocation if appropriate.

If you wish to apply, please send a full C. V. to the Personnel Manager, Beecham Pharmaceuticals Research Division, Medicinal Research Centre, Coldharbour Road, The Pinnacles, Harlow, Essex CM19 5AD. Alternatively, if you would like an application form, please telephone our 24-hour answering service on (0279) 419373 quoting Ref. 12/87. For further information, please telephone the Personnel Manager on (0279) 29631 Ext. 201.

(2408)A

Beecham
Pharmaceuticals

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nature

THE INTERNATIONAL JOURNAL OF SCIENCE

needs a

biological scientist

chiefly to assist with the selection and editing of research papers submitted for publication, although contributions to other parts of the journal will be encouraged. The post could be held in either London or Washington, but in the latter case it will be necessary to spend some time in the London office first.

Essential qualifications:

- First-hand experience of research up to PhD level
- Knowledge of the frontiers of biological research
- Broad general interest in science
- Evidence of literacy

Applications should consist of a full *curriculum vitae*, a brief statement of the applicant's views of *Nature* and how he or she would expect to contribute to it and the names of two referees. They should be sent to the Editor, *Nature*, at 4 Little Essex Street, London WC2R 3LF, UK or 1137 National Press Building, Washington DC 20045, USA, to arrive not later than 3 April 1987.

(2390)A

FU BERLIN

An der Freien Universität Berlin ist folgende Stelle zu besetzen:

Fachbereich Natur- und sozialwissenschaftliche Grundlagenmedizin und medizinische Ökologie

INSTITUT FÜR MOLEKULARBIOLOGIE UND BIOCHEMIE

PROFESSOR/IN (STIFTUNGSPROFESSUR)

Molekularbiologie und Biochemie der Entwicklung C4

Aufgabengebiet: Die Stiftungsprofessur ist auf drei Jahre begrenzt und für diese Zeit als Forschungsprofessur ausgewiesen. Molekularbiologie und Biochemie der Entwicklung sollen als Fach in der Forschung vertreten werden. Anschließend erfolgt Überleitung in die Nachfolge einer planmäßigen C-4-Professur. Mit Übernahme in die planmäßige Professur treten Aufgaben in der Lehre (Durchführung der Lehrveranstaltungen gemäß Approbationsordnung für Ärzte im Fach Physiologische Chemie) hinzu. Das Fach Molekularbiologie und Biochemie ist in Forschung und Lehre zu vertreten.

Einstellungsvoraussetzungen: Habilitation für Molekularbiologie oder Biochemie oder der Habilitation gleichwertige wissenschaftliche Leistung.

Erwünscht: Lebensalter nicht über 45 Jahre. Bewerbungen mit den üblichen Unterlagen sind innerhalb von 6 Wochen unter Angabe der Kennziffer 3/345 117 zu richten an die Freie Universität Berlin, Fachbereich Natur- und sozialwissenschaftliche Grundlagenmedizin und medizinische Ökologie, Gustav-Meyer-Straße 7, D-1000 Berlin 33.

(W3916)A

RESEARCH ASSOCIATE/ POSTDOCTORAL FELLOW

A position is available to investigate the localization and expression of ion channels in skeletal muscle. We are studying the neural control of these processes during development and in adult muscle. The primary focus is upon sodium channels, acetylcholine receptors, and chloride channels. Techniques employed include conventional electrophysiological recordings, loose and tight patch voltage clamp, vibrating extracellular microelectrodes, immunocytochemistry, and *in situ* hybridization with RNA probes. Send c.v., statement of research interests and the names of three references to **Dr John H. Caldwell, Dept. of Molecular and Cellular Biology, National Jewish Center, 1400 Jackson St., Denver, CO 80206 or Dept. of Physiology, Univ. of Colorado Medical School, Denver, CO 80262.** (NW645)A

UNIVERSITY OF CINCINNATI COLLEGE OF MEDICINE

Pre- and postdoctoral positions

are available in pharmacology for those students who are interested in training in the molecular characterization of muscle, pumps and channels. In particular, our research efforts are directed towards regulation and development of muscle. This training offers a unique opportunity to combine contemporary pharmacology with molecular genetics. The future of pharmacology lies in an understanding of the molecular control of macromolecules. Employment opportunities are at a very high level and include both academic careers and scientific and managerial positions in the pharmaceutical industry. Interested parties should contact either Arnold Schwartz, PhD, or Jeffrey Robbins, PhD, at the University of Cincinnati College of Medicine, Department of Pharmacology, 231 Bethesda Avenue, Cincinnati, Ohio, 45267-0575.

Equal Opportunity/Affirmative Action Employer.

(NW640)A

UNIVERSITY COLLEGE LONDON

School of Medicine

BIOCHEMIST/ PHARMACOLOGIST

Applications are invited for a postdoctoral Biochemist/Pharmacologist to work in the laboratories of medical oncology and toxicology in the School of Medicine, University College London. The project concerns mechanisms of cell killing by anti-cancer drugs and protection by intra-cellular thiols. Previous experience in cell biology and/or DNA structure is desirable. Post is for 3 years, salary scale University IA. Applications to Dr Robert Souhami, Faculty of Clinical Sciences, University College London, University Street, London WC1. Closing date: 9 April 1987. (2363)A

UNIVERSITY OF CAMBRIDGE

Applications invited for the

PROFESSORSHIP OF ORGANIC CHEMISTRY

(1702)

tenable from 1 October 1988

Present pensionable stipend £22,085 (under review).

Applications, marked 'Confidential', with the names of two referees should be sent to the Secretary General of the Faculties, from whom further information may be obtained, at the General Board Office, The Old Schools, Cambridge, CB2 1TT. Closing date: 29 May 1987. (2375)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF BIOCHEMISTRY

RESEARCH ON SUGAR TRANSPORT PROTEINS

Our discovery that bacterial sugar transport proteins are homologous to the human glucose transporter (*Nature*, **325**, 641, 1987) will be exploited to elucidate the molecular mechanism of an apparently ubiquitous nutrient capture process. A 3-year project funded by the SERC protein engineering initiative involves sequencing related transport genes, *in vivo* and *in vitro* mutagenesis, recombinant DNA techniques, protein chemistry, immunochemistry, etc. Applicants for a postdoctoral post (Cambridge RA age-related scales £8,020—£11,790) or postgraduate studentships may be qualified in biochemistry, genetics, microbiology or allied subjects. Send full c.v. plus names of two referees as soon as possible to Dr Peter Henderson, Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QW. (2402)A

UNIVERSITY OF BIRMINGHAM

FACULTY OF MEDICINE AND DENTISTRY

Department of Clinical Chemistry

Sample preparation for DNA analysis

A Research Associate 1B is required to join an expanding molecular biology group to wor on methods for the automatio of DNA probe-based diagnosti tests. The successful applicant wil investigate sample preparatio handling procedures using variety of clinical specimens. He she will also liaise with a grou of engineers on aspects of wor simplification and automation.

Applicants should possess good honours degree in a relevan biological subject. Experience o molecular biology technique would be an advantage but is no essential as training will be given The salary is for up to two year and will be in the range £7,055 £9,495 with superannuation.

Applications (3 copies) including a curriculum vitae and the names of two academic referees should be sent to: The Assistant Registrar, The Medical School, Birmingham B15 2TJ by 9 April 1987. Quote Ref: RA/CC/LJK.

An equal opportunities employer. (2388)A

IMPERIAL COLLEGE

(University of London)

CENTRE FOR REMOTE SENSING

A Research Officer is required to provide Image Processing user support for the Centre facilities which include VAX 11/780 12S and PDP/11 12S systems. The successful candidate will work with the Computer Manager and should have comprehensive competence in image processing and operation of the systems. In addition, the Research Officer will be expected to train and demonstrate to inexperienced students and staff from a variety of disciplines, assist with software development and participate in installation of new hardware and software.

Salary will be in the range £9,413—£14,173 or £13,673—£17,093 (under review) inclusive of London Allowance, according to qualifications and experience.

Further particulars from and applications with the names of two referees to: Prof. J.L. Knill, Chairman, Centre for Remote Sensing, Imperial College of Science and Technology, The Blackett Laboratory, Prince Consort Road, London SW7 2BZ. Tel. 589 5111 Ext. 5501. (2389)A

UNIVERSITY OF ABERDEEN

Department of Surgery

POSTDOCTORAL RESEARCH FELLOW

uired for a three year grant-
ded project to generate human
noclonal antibodies against
in tumours. Applicants with
perience in immunology/tissue
ture preferred. Starting date as
on as practicable.

Salary within the range £8,020 -
,000 pa on Range IA Scale for
search and Analogous Staff
nder review).

Further particulars and applica-
n forms from The Personnel
fice, The University, Regent
alk, Aberdeen AB9 1FX with
om applications (2 copies)
ould be lodged by 10 April 1987
ef: ER/057). (2365)A

UNIVERSITY LABORATORY OF PHYSIOLOGY OXFORD

TWO POSTDOCTORAL RESEARCH POSITIONS

ailable to work on (1) the ionic
annels of pancreatic B-cells and
) carotid body cells. Applicants
th patch-clamp experience
ongly preferred. (1). One year
th possibility of further renewal.
). Three years. Both salaries will
on the RS1A scale.

Written applications, including
curriculum vitae and the names
d addresses of two referees
ould be sent to the Admin-
rator, University Laboratory of
ysiology, Parks Road, Oxford
X1 3PT. Written or phoned
quiries may be made to (1) Dr F
Ashcroft (0865) 272456 or (2)
P C G Nye 272433. (2367)A

CHARING CROSS AND WESTMINSTER MEDICAL SCHOOL

(University of London)

DEPARTMENTS OF IOCHEMISTRY AND ONCOLOGY

TWO RESEARCH ASSISTANTS

pplications are invited for two
osts of research assistant (at least
ne at postdoctoral level) funded
y a three-year grant from the
ancer Research Campaign to
ichard Begent and Alan Mal-
olm. This will involve a system-
tic study of altered gene expres-
on in colon cancer. Salary up to
0,375 + London Allowance
nder review).

Applications on forms obtain-
ble from The Secretary, Charing
ross and Westminster Medical
chool, The Reynolds Building, St
unstan's Road, London W6
RP, to be submitted within two
eeks of the appearance of this
lvertisement. (2370)A

The German Collection of Microorganisms (DSM) is part of the Gesellschaft für Biotechnologische Forschung mbH, a large research institution funded by the Federal Government and the State of Lower Saxony. During 1987 the DSM will expand its activities, and as a result a number of scientific positions are available in the following areas:

1. Archaebacteria/phototrophic bacteria
2. Pathogenic bacteria (medical microbiologist)
3. Bacteria for quality control and plasmids
4. Genetically modified bacteria
5. Chemotaxonomy and identification of bacteria
6. Public relations work, further educational courses, and identification.

In addition to the duties of the culture collection successful candidates will be expected to carry out relevant research. Applicants should have appropriate experience.

The positions are available from about 1.9.1987. The place of work will be Braunschweig. All positions are initially limited until 31.12.1989.

Salaries will be within the Federal employees scale (BAT). The normal benefits offered to public employees also apply.

Applications with c.v., list of publications, a recent photograph, and copies of degree certificates etc. to:

Personalabteilung
Gesellschaft für Biotechnologische Forschung mbH
Mascheroder Weg 1
D-3300 Braunschweig
Federal Republic of Germany

(W3926)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitbly qualified men and women for appointment to the following positions:

RESEARCH SCHOOL OF PHYSICAL SCIENCES

POSTDOCTORAL FELLOW/ RESEARCH FELLOW IN THEORETICAL PHYSICS

The Department (Head: Dr B.A. Robson) is engaged in research into theoretical aspects of nuclear physics, elementary particles and field theory, statistical mechanics, transport processes, solid state physics, atomic and molecular physics and plasmas. In this instance, preference will be given to applicants in the field of atomic and molecular physics with special interests in *ab initio* calculations of electron scattering and photon absorption from atoms and molecules. Closing date: 30 April 1987. Ref: PS.25.2.1.

Faculty of Science

DEPARTMENT OF PHYSICS AND THEORETICAL PHYSICS

LECTURER IN PHYSICS

A tenurable lectureship is available in the Department of Physics and Theoretical Physics. The successful applicant will be required to contribute to both graduate and undergraduate teaching programs and to participate in experimental research in the Department's shock-tube laboratory. Current research programs are concerned with shock-surface interaction, ionising shock stability, collision processes, radiation processes, and hyper-velocity aero-thermodynamics in free-piston shock-tunnel facilities. Other research interests of the Department are in laser physics, quantum optics and nuclear structure physics. Applicants should hold a relevant PhD degree and be able to take up duty without delay. Closing date: 17 April 1987. Ref: FS.25.2.1.

CONDITIONS OF APPOINTMENT: Salary will be in accordance with qualifications and experience within the range: Postdoctoral Fellow Grade 1 (at a fixed point) $A24,013—$27,507 p.a.; Research Fellow $A27,859—$36,600 p.a.; Lecturer $A27,859—$36,600 p.a. Appointment, unless otherwise stated, will be: Research Fellow normally up to three years, with the possibility of extension to maximum of five years; Postdoctoral Fellow normally two years, with the possibility of extension to maximum of three years. Lecturer will be for four years in the first instance with the possibility, after review, of appointment to retiring age. Grants are provided towards travel and removal, though special conditions may apply to persons appointed to externally funded positions. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Applicants should quote the advertisement reference when requesting further particulars and when submitting applications, which should be forwarded to the Registrar, Australian National University, GPO Box 4, Canberra, ACT 2601, Australia; further particulars also available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. The University is an equal opportunity employer.

(W3915)A

UNIVERSITY OF GLASGOW

WELLCOME UNIT OF MOLECULAR PARASITOLOGY

Postdoctoral Scientists

(various levels, Range 1A)

Research Assistants

(Range 1B)

Research Technicians

(various grades)

The Wellcome Trust has established this Unit to study the basic biology of parasites, utilizing cellular, biochemical, genetic and molecular technology. The Unit, located in newly refurbished laboratories in the University Departments of Veterinary Parasitology and Genetics, will investigate specifically differentiation, development, growth regulation and genetics of the protozoan parasites *Trypanosoma* and *Theileria* and the nematode *Ostertagia*. These parasites display unique features of interest. There will be a high degree of interfacing with surrounding University Departments with parasitological and molecular expertise.

Positions are available, for 3 or 5-year terms in the first instance, at various levels of seniority in the above categories. Starting dates are from 1 April-1 October 1987. Applicants, preferably with a background in biochemistry/molecular biology, should send a c.v. with names of 2 referees to **Dr A. Tait (Director) and Dr J.D. Barry, Institute of Genetics, Church Street, Glasgow G11 5JS.** For further information, phone 031-667 1081 Ext. 3529 (Dr Tait) or 041-339 8855 ext 6215 (Dr Barry). (2376)A

— IRELAND —

Agricultural Inspector (Toxicologist)

DEPARTMENT OF AGRICULTURE

Essential: A degree in **medicine or veterinary medicine or** a first or second class honours degree, or equivalent, in the final examination for which **pharmacy** or **pharmacology** was taken as a major subject **or** a first or second class honours degree in **agricultural science** or a first or second class honours degree in the final examination for which a subject relevant to the hazard assessment of toxic chemicals e.g. **biochemistry, chemistry, biology, physiology or microbiology** was taken as a major subject and a postgraduate qualification in pharmacology, epidemiology or statistics;

- a postgraduate qualification in occupational medicine and toxicology, or in toxicology;
- five years postgraduate experience in hazard assessment of toxic chemicals, preferably pesticides, including experience of histopathological laboratory equipment and techniques.

Salary will be in the range: IR£16,669—IR£23,292.

Upper age limit: 50 years.

Closing date: 30 April, 1987.

Application forms and further particulars are available from the Secretary, Civil Service Commission, 1 Lower Grand Canal Street, Dublin 2.

THE CIVIL SERVICE COMMISSIONERS ARE COMMITTED TO A POLICY OF EQUAL OPPORTUNITY. (2366)A

POSTDOCTORAL OR RESEARCH ASSOCIATE

Postdoctoral or Research Associate (Electrophysiologist-Neuropharmacologist) position available immediately for research on neurotransmitters involved in the circuitry controlling the membrane potential of motoneurons during sleep and wakefulness. Experience in intracellular recording in cat CNS. We are particularly interested in studying the transmitters involved in the supraspinal and local circuit control of motoneurons during active (REM) sleep in adult cats. Contact **M. Chase, Department of Physiology, UCLA Center for the Health Sciences, Los Angeles, CA 90024-1751.** *The University is an equal opportunity/affirmative action employer.*

(NW656)A

POSTDOCTORAL OR RESEARCH ASSOCIATE

Postdoctoral or Research Associate (Neuroanatomist) position available immediately for research on anatomical and electrophysiological studies of sleep and wakefulness and of aging motoneurons in the old cat. Experience in quantitative evaluation of the anatomy of functionally defined neurons and neuronal connections. We are studying the electrophysiological properties of these cells by recording intracellularly in both acute experiments and in the chronically prepared cat during sleep and wakefulness. We wish to provide an anatomical basis for our electrophysiologically derived data. Contact: **M. Chase, Department of Physiology, UCLA Center for Health Sciences, Los Angeles, CA 90024-1751.** *The University is an Equal Opportunity/Affirmative Action Employer.*

(NW657)A

ΠΡΟΒΛΕΨΗ ΝΕΩΝ ΒΕΣΕΩΝ ΣΤΟ ΙΑΤΡΙΚΟ ΤΜΗΜΑ ΤΟΥ ΠΑΝΕΠΙΣΤΗΜΙΟΥ ΚΡΗΤΗΣ

Το Ιατρικό Τμήμα του Πανεπιστημίου Κρήτης προτίθεται να προκηρύξει μέσα στο 1987 νέες θέσεις στους εξής Τομείς:

(1) Βασικών Επιστημών, (2) Εργαστηριακής Ιατρικής, (3) Μορφολογίας, (4) Ακτινολογίας, (5) Ψυχιατρικής και Επιστημών Συμπεριφοράς, (6) Κοινωνικής Ιατρικής, (7) Νευρολογίας και Αισθητηρίων Οργάνων, (8) Παθολογίας, (9) Μητέρας—Παιδιού (Παιδιατρικής, Γυναικολογίας, κ.λ.π.), (10) Χειρουργικής.

Το Πανεπιστήμιο θα καταβάλει κάθε προσπάθεια για να προσελκύσει το καλύτερο επιστημονικό προσψπικό που διαθέτει η χώρα μας στο εξωτερικό και στο εσωτερικό. Η Ελληνική υπηκοότητα είναι απαραί τητο προσόν.

Οσοι ενδιαφέρονται μπορούν να γράψουν στη Διδα Ρένα Μαρκάτζη, Ιατρικό Τμήμα Πανεπιστημίου Κρήτης, Ηράκλειο—Κρήτης (τηλ.: 081-234010 ή 234307, εσ. 54). Κατά την προκήρυξη πληροφορίες θα σταλούν επίσης στις Ελληνικές βείες και Προξενεία του Εξωτερικού. (W3897)A

UNIVERSITY OF THE WEST INDIES

Cave Hill Campus, Barbados

SENIOR LECTURER/ LECTURER AND LECTURER/ASSISTANT LECTURER IN COMPUTER SCIENCE

DEPARTMENT OF MATHEMATICS

Senior Lecturer/Lecturer: Applicants should be competent to teach i) Programming Languages; ii) Design and Analysis of Algorithms; AND/OR i) Computer Networks; ii) Data Base Management Systems. Other areas of interest include Operating Systems; Software Engineering; Modelling and Simulation; Computer Architecture; Compiler Design; Interactive Computer Graphics; Numerical Analysis.

Lecturer/Assistant Lecturer: Applicants should be competent to teach two or more of the following: Programming Languages; Design and Analysis of Algorithms; Computer Networks; Data Base Management Systems; Operating Systems; Software Engineering; Interactive Computer Graphics; Modelling and Simulation; Compiler Design and Numerical Analysis. Applicants with other active interests in Computer Science will also be considered.

The successful applicants will be expected to assume duties by 1987 or as soon as possible thereafter.

Salary Scales (under reviev Senior Lecturer: BDS$41,976 1368 — 50,184 × 1464 — 54,5 (Bar) × 1464 — 57,504 p.a., Lecturer: BDS$31,644 × 1368 39,852 (Bar) × 1368 — 48,0 p.a., Assistant Lecture BDS$26,112 × 1272 — 28,656 p. Up to five full economy class pa sages plus baggage allowance US$1200 on appointment and nc mal termination. Special all wance up to US$400 for shipme of academic books and teachin research equipment on appoin ment. Unfurnished accommod tion at 10% of basic salary; optional housing allowance 20% of basic salary to staff makin own housing arrangements. UW contribution of equivalent of 10 of basic salary to Superannuatic Scheme. Annual Study and Trav Grant for self, spouse and up three children. Book Grant up BDS$600 per annum.

Detailed applications (thre copies) giving full particulars qualifications and experienc date of birth, marital status ar the names and addresses of thre (3) referees should be sent as soc as possible to the CAMPUS REG ISTRAR, UNIVERSITY O THE WEST INDIES, P.O. Bo 64, BRIDGETOWN, BARBA DOS. The University will sen further particulars for these pos to all applicants. These particular may also be obtained from th Association of Commonwealt Universities (Appts), John Foste House, 36 Gordon Square, Lon don WC1H 0PF. (W3929)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL

(University of London)

RESEARCH TECHNICIAN

ACADEMIC DEPARTMENT OF HISTOPATHOLOGY

The Department requires a technician experienced in standard histological techniques. Most of the work will involve immunocytochemistry, for which training is available. The successful candidate should have a good honours degree in a biological science, and should be innovative and able to contribute to departmental research projects with the minimum of supervision. This is a permanent position presently funded at the T5 level, £7,274—£8,588 plus £840 London Allowance, with the possibility of promotion.

Further particulars and an application form from the Personnel Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. 01-672-1255 ext 5481. Closing date 9 April 1987. Quote reference 29/87.

(2394)A

THE UNIVERSITY OF MANCHESTER

POSTDOCTORAL RESEARCH ASSOCIATE IN RHEUMATOLOGY

Applications are invited for thi post, to work on a project funde by the Arthritis and Rheumatism Council, to study the nature o heat-shock antigens recognised b antibodies from patients wit certain types of rheumatic disease The laboratory also has an interes in other aspects of autoimmunit in human disease. Candidates wit experience in molecular or ce biology, biochemistry or immuno logy are particularly encourage to apply.

Further information can b obtained by telephoning D Robert Bernstein on (061) 27 4357. Salary range pa (unde review) £8,020 - £12,780. Applicants should send a full curriculu vitae with the names and addresse of two referees to: Dr R M Bern stein, Department of Rheum atology, Medical School, Th University, Manchester M13 9PT

(2397)A

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

Department of Entomology

LECTURESHIP IN THE BEHAVIOUR OF DISEASE VECTORS

plications are invited for this w position established under the niversity of London's Academic itiatives Scheme, for three years the first instance. The successful plicant will be responsible for aching and research in the Deartment of Entomology and will ork in collaboration with the epartment of Pure and Applied iology at Imperial College where /she will hold an Honorary ectureship.

Preference will be given to aplicants who have undertaken rearch on the behaviour of disease ectors under natural conditions in e field.

The appointment will be on the alary scale for non-clinical lecrers; £8,020 to £15,700 plus 1,393 London Weighting (under view), with membership of USS.

For further particulars, please ontact the Personnel Office, ondon School of Hygiene and ropical Medicine, Keppel Street, ondon WC1E 7HT (Tel: 01-636-636), to whom applications with urriculum vitae and the names of ree referees should be sent by 10 pril 1987. (2411)A

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nature classified

LONDON OFFICE
4 Little Essex Street
London WC2R 3LF
Tel: (01) 240-1101
Telex: 262024

NEW YORK OFFICE
65 Bleecker Street
New York, NY 10012
Tel: (212) 477-9600

TORONTO OFFICE
Peter Drake & Associates
17 Pine Crescent
Toronto, Ontario M4E 1LI
Tel: (416) 690-2423

SAN FRANCISCO OFFICE
Suite 1408
582 Market Street
San Francisco
CA 94106 (415) 781-3803, 3804 or 3801

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

Department of Anatomy and Biology as Applied to Medicine

A RESEARCH ASSISTANT

preferably post-doctoral, is required to work on the development, in the mouse, of asymmetry and handedness, particularly of the heart, starting 1 October 1987, or earlier. The project is funded by The Wellcome Trust for three years.

Experience in cell or developmental biology is desirable. Recent graduates, or those about to graduate, will also be considered.

Applications with the names of two referees to: Professor L Wolpert, Department of Anatomy and Biology as Applied to Medicine, The Middlesex Hospital Medical School, Cleveland Street, London W1P 6DB. (2405)A

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

Department of Medical Microbiology

RESEARCH FELLOW

Applications are invited from a scientist with MSc or a good first degree and experience in virology and/or biochemical or molecular biological techniques to join a three-year Wellcome Trust research project on viral hepatitis.

The appointment will be at an appropriate point on Research and Analogous scale 1B or 1A according to age, qualifications and experience.

Applications with full curriculum vitae and the names of two referees should be sent to the Personnel Office, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT by 30 April 1987 quoting ref KT1. (2410)A

UNIVERSITY OF LONDON

Institute of Neurology

Applications are invited for a

POST-DOCTORAL POSITION

available for 2 years in the first instance in an MRC programme grant group monitoring transmitter amine metabolism in the freely moving rat. Techniques are being applied in studies of feeding, stress and animal models of neurological and psychiatric illness.

Applicants should have expertise in relevant aspects of experimental psychology and be interested in neurochemistry and/or pharmacology. Initial salary within range £9,413 - £11,768 (under review).

Apply with CV and names of two referees to: Professor G Curzon, 1 Wakefield Street, London, WC1N 1PJ within three weeks of the appearance of this advertisement. (2404)A

Expert knowledge of electrophysiology

Patch clamp and microelectrodes

Applications are invited for a research scientist position with a leading french drug company that is located near PARIS.

Candidates should have an expert knowledge of electrophysiology (patch clamp an microelectrodes). They should have also a knowledge of data processing and signal treatment.

Candidates should hold a Ph. D or an equivalent degree and have substantial experience.

Applicants submit their C.V. with statement of major research interest under ref 4616/NA, to

MEDIA PA.

71, avenue Victor-Hugo - 75116 PARIS, FRANCE, who will forward.

(W3920)A

SANDWELL HEALTH AUTHORITY

Midland Centre for Neurosurgery and Neurolgy,
Holly Lane, Smethwick, Warley
West Midlands, B67 7JX

DEPARTMENT OF PATHOLOGY

ELECTRON MICROSCOPIST IN CHARGE OF SECTION

(Basic/Senior Science Graduate)

required on a Locum basis (available grading up to Senior as defined by Whitley Council PTA Regulations). Well housed department with two other staff, two Siemens microscopes, several ultramicrotomes. Successful applicant will be required to continue and develop service in Muscle, Central and Peripheral Nervous System Pathology, to Hospital and Region. Collaboration with current research projects will be expected and there is an excellent opportunity to develop a personal research programme on the readily available material.

Intending applicants are welcome to visit by appointment with Dr M V Salmon (Neuropathologist) 021.558.3232 ext. 314.

Job description and application form from the Administrator, Midland Centre for Neurosurgery and Neurology at the above address.

Closing date 31st March 1987.

Sandwell is a non smoking Health Authority. (2399)A

McLAUGHLIN RESEARCH INSTITUTE

BIOMEDICAL RESEARCHER

PhD or MD established investigator with grant support to head research program at superb facility. Institute has 20 year tradition of NIH-funded support in immunology, with established 8000 mouse colony — many H-2 recombinant lines. Fine quality of life. Details upon request. Please submit c.v., 3 references and research interests to: **McLaughlin Research Institute 1625 3rd Avenue North, Great Falls, Montana 59401 Ph: 406-761-5833**. Application by May 30. *Equal opportunity/AA Employer.* (NW655)A

POSTDOCTORAL NEUROBIOLOGIST

An opening is available to study chemosensory transduction mechanisms in vertebrate taste cells. Candidates must be familiar with microelectrode recording techniques. Salary will be commensurate with experience and will be comparable to NIH levels. The successful candidate will participate in a new college-wide program in Neurobiology at CSU. Send c.v., letter, and names of references to **Dr Stephen Roper, Professor and Chairman, Department of Anatomy, Colorado State University, Ft. Collins, CO, 80523**. EO/AA employer. (NW654)A

UNIVERSITI SAINS MALAYSIA

School of Industrial Technology

Applications are invited for the post of

Professor in Food Technology

Qualifications and Experience: Candidates should have a Masters or PhD degree with several years of teaching and/or research experience in any of the following areas:

Food Processing and Manufacture
Food Engineering
Biotechnology as applied to food production and manufacture
Treatment/Utilisation/Disposal of food processing wastes

Salary and Allowance: Salary and entertainment allowances will range from M$51,720 to M$58,920 per annum. In addition, candidates will also be provided furnished accommodation up to the value of M$1,400 per month. Candidates will also be paid gratuity at 17½% of last drawn basic salary for each completed month of service at the end of the contract.

Other Benefits: Return passage for appointee and family (wife and 3 children); excess baggage assistance; 30 days paid annual leave, free medical benefits for staff and family.

Provisions for Research: Selected candidates will also be allocated an initial capital sum of M$250,000 for purchase of equipment for research and also a research budget of $20,000 to employ a research assistant and to purchase consumables for research work.

Tenure: Selected candidate will be appointed on contract for 3 years initially which is renewable.

Detailed curriculum vitae with complete list of research publications should be sent to Registrar, Personnel Section, Universiti Sains Malaysia, 11800 USM PENANG, Malaysia. Enclose personal biodata and references. (W3886)A

UNIVERSITY OF LEICESTER

Department of Biochemistry

TECHNICIAN GRADE 3

Applications are invited for the above post which is available immediately within a group working on the organisation and expression of enkaryotic genes. The majority of the work will involve mammalian cell culture technology, including monoclonal antibody production and recombinant DNA transfection. Although experience in these fields would be an advantage, it is not essential. Minimum qualifications ONC, TEC or equivalent.

Salary on the scale £5,968 to £7,040 a year.

Applicants should send a curriculum vitae and the names and addresses of two referees to Mr J A Mackley, Departmental Superintendent, Department of Biochemistry, University of Leicester, University Road, Leicester, LE1 7RH. Closing date is 12 April 1987. (2401)A

UNIVERSITY OF LEICESTER

Department of Botany

POSTDOCTORAL RESEARCH ASSOCIATE IN PLANT GENETIC ENGINEERING

A research associate is required to join a small team working on the genetic manipulation of crop plants. The work is specifically concerned with the genetic transformation of pulse legumes and the construction of gene fusions with developmentally regulated promoters. The ideal candidate would have experience in plant tissue culture, genetic transformation procedures and molecular biology. Familarity with recombinant DNA technology would be useful, but not essential.

The post is available immediately and is for two years in the first instance, with the possibility of a further year. The appointment will be on the postdoctoral research associate RA1A scale. Salary, depending on age and experience, will be in the range of £9,303 to £12,603 pa (Salary scales subject to conformation).

Applications should be sent to: Dr J Draper, Department of Botany, University of Leicester, University Road, Leicester, LE1 7RH, together with a cv and the [illegible] and addresses of two ref[illegible]losing date is 30 April (2384)A

FINSEN LABORATORY, COPENHAGEN

PLASMINOGEN ACTIVATION AND CANCER RESEARCH FELLOW

is sought for investigations of the role of the plasminogen activation system in invasive growth and tissue destruction in cancer. The position is funded by the The Danish Cancer Society until the end of 1988, with the possibility of extending the length of employment. The person will join a research team consisting at present of 7 scientists and a corresponding number of technicians, and will be engaged in one of the following current projects:

□ The isolation of the receptor for urokinase-type plasminogen activator (u-PA) and its functional and structural characterization. Development of monoclonal antibodies against the receptor.

□ Investigations of the biological function of u-PA and the enzyme's receptor-binding, using model systems for degradation of extracellular matrix proteins and invasiveness.

□ Investigation of the activation mechanism for the proenzyme to u-PA.

□ Immunohistochemical identification of the components of the PA-system in tumors and in normal tissue.

□ Identification of mRNA for the components of the PA-system, by *in situ* hybridization.

□ Investigation of hormone- and growth factor regulation of the synthesis of a PA-inhibitor, by the use of cDNA, genomic DNA and the CAT-technique.

The candidate must have a PhD degree in biochemistry or biology, or the equivalent. Experience within one or more of the fields of protein chemistry, immunochemistry, cell culture and model systems for invasiveness, receptor studies, enzyme kinetics, immunohistochemistry and gene technology is an advantage.

The Finsen Laboratory is a cancer-research laboratory staffed with approximately 40 scientists and technicians. It has modern equipment and optimal auxilliary facilities. At present, the research is concentrated on three main areas: the role of the plasminogen activation system in invasive growth and tissue degradation, and flow cytometric and cytogenetic investigations of cancer.

Salary range: 150,000—220,000 D.kr. Further information can be obtained from Keld Danø, Medical Director, The Finsen Laboratory, Rigshospitalet, Strandboulevarden 49, DK-2100 Copenhagen Ø., Denmark, tel. 01-260850, ext. 215. Please address application to the Board of Directors of Rigshospitalet and send it no later than April 15 to the Finsen Laboratory. (W3927)A

UNIVERSITY OF NOTTINGHAM CANCER RESEARCH CAMPAIGN LABORATORIES

POSTDOCTORAL APPOINTMENT

Applications are invited for Po doctorate appointments to jo Professor R.W. Baldwin's grou investigating the use of monoclonal antibody conjugates fo cancer treatment. Applicant should have qualifications in on of the biological sciences. Th research will involve the *in vitr* and *in vivo* assessment of antibod conjugates. There will also be a opportunity to collaborate in clinical studies.

Salary will be in the range (1B).

Applications, including curriculum vitae and names and addresse of two referees should be sent to The Secretary, Cancer Research Campaign Laboratories, University of Nottingham, Nottingham NG7 2RD. (2373)A

THE UNIVERSITY OF MANCHESTER

RESEARCH ASSISTANT IN MEDICAL GENETICS

Applications are invited for this post, tenable for up to three years from August 1, 1987, to work with Dr T Strachan and Professor R Harris on a project investigating the molecular genetics of haemochromatosis. Appointee will be encouraged to register for a higher degree.

Applicants should hold or expect to receive a good honours degree in an appropriate subject and preference will be given to candidates with a strong background in molecular biology. Initial salary £7,055 pa (under review). Superannuation. Enquiries, and applications naming two referees, should be addressed to Dr T Strachan, University Department of Medical Genetics, St Mary's Hospital, Manchester M13 0JH. (061 276 6122). (2395)A

POSTDOCTORAL POSITIONS

available to study structure and function of murine (MuLV, SFFV) and human (HIV) retroviruses. Projects involve: (1) site-specific mutagenesis of cloned viral genes — experience in recombinant DNA methodologies required; (ii) immunochemical studies of viral proteins — experience in monoclonal antibody production and protein characterization desired. Send curriculum vitae and names of three references to: **Dr A. Pinter, Laboratory of Retroviral Biology, The Public Health Research Institute, 455 First Avenue, New York, NY 10016.** (NW648)A

LIVERPOOL POLYTECHNIC
Department of Biology
LECTURER II/SENIOR LECTURER IN MICROBIOLOGY
LII/SL £8,595 — £15,873

Applications are invited for this post which is available from 18th April 1987 or as soon as possible thereafter. Applicants should have a good honours degree in a biological science and have or shortly expect to gain a PhD in a related area. The post will involve teaching microbiology at undergraduate level with a major input into the continuing development of biotechnology. Experience in microbial physiology, especially fermentation technology, will be an advantage. The appointee will be expected to develop an active research programme.

For further particulars and an application form contact the Personnel Officer, Liverpool Polytechnic, Rodney House, 70 Mount Pleasant, Liverpool L3 5UX (Tel. 051 207 3581 Ext. 2518/2519) to whom applications should be returned not later than 3rd April, 1987.

Liverpool Polytechnic is an Equal Opportunity employer and welcomes applications irrespective of race, sex, marital status or disability. (2400)A

ASTON UNIVERSITY
Diabetes Research
CONTRACT RESEARCH ASSISTANT/OFFICER IN BIOCHEMISTRY/ ENDOCRINOLOGY

n interesting research position ists for a well qualified graduate esearch Assistant) or recently ualified postdoctoral scientist esearch Officer) to join a rearch group, in the Department f Molecular Sciences, investigatg hormonal aspects of diabetes ellitus. The appointee will enage in a study of the interaction of iguanides and insulin in the conol of glucose uptake and etabolism.

The post is for one year in the first instance with possible renewal for a further one or two years. Commencing salary will be within the range £7,055 to £9,495 per annum (presently under review), depending upon qualifications, age and experience.

Application forms and further particulars may be obtained from the Personnel Officer (Academic Staff), quoting Ref: 8714/6, Aston University, Aston Triangle, Birmingham B4 7ET. Tel: 021 359 8070 (24-hour answerphone). Closing date for the receipt of applications is 10th April 1987. (2412)A

BIOTECHNOLOGISTS!

What's going on in the world's fastest-growing scientific discipline?

To find out, send for a sample copy of BIO/TECHNOLOGY, the International Monthly for Industrial Biology, to

Felicity Lyons, Macmillan Journals, 4, Little Essex St.
London, WC2R 3LF

or

Paige Beaver, Bio/Technology, 65, Bleecker St.,
New York, NY10012, USA

UNIVERSITÄT KARLSRUHE
Postdoctoral Position

At the Institute for Genetics

A postdoctoral position is available to join a group studying expression of the albumin and vitellogenin genes of Xenopus. Applicants should have a PhD in biochemistry or biology and preference may be given to those with experience in recombinant DNA research, protein fractionation and cell culture techniques.

Please send a curriculum vitae and the names of two referees to Dr G.U. Ryffel, Institut für Genetik, Postfach 3640, D-7500 Karlsruhe 1. (W3924)A

FRIEDRICH MIESCHER-INSTITUT

Applications are invited for the position of a

GROUP LEADER IN PLANT BIOLOGY

Candidates should have a strong research background in Plant Sciences at the cell and/or molecular level, as well as a demonstrated ability to develop an independent research programme and to lead a group.

The candidate will be expected to carry out research in an area complementing the work of five other group leaders: Witek Filipowicz (gene expression and RNA processing), Barbara Hohn (genetic flux in plants), Thomas Hohn (molecular plant virology), Patrick King (a genetic approach to plant hormones), and Frederick Meins (plant development). The groups each consist of postdoctoral fellows, graduate students and technical staff, and occupy independent laboratories.

We would prefer to make the appointment at the level of a senior group leader, but an appointment at a more junior level is not excluded.

A curriculum vitae and a list of publications should be submitted before March 31st, 1987 to: **Search Committee (Plant Group Leader), Friedrich Miescher-Institut, PO Box 2543, 4002 Basel, Switzerland.** (W3890)A

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INSTITUTE OF HORTICULTURAL RESEARCH
SHORT-TERM SCIENTIFIC OFFICERS POSTS

IHR — LITTLEHAMPTON requires

(1) A Scientific Officer (Band II) to join a team studying the biological control of white rot disease of onion. Ref 1/L.

(2) A Scientific Officer (Band II) with strong interests in plant physiology to join the Crop Production Division to have day to day responsibility for experiments on the effects of high relative humidities on plant growth and development and on the causes of plant to plant variation in pot chrysanthemums. Ref 2/L.

IHR — WELLESBOURNE requires

(1) A Plant Pathologist Scientific Officer (Band I) to work on a programme of research on cavity spot disease of carrots caused by Pythium spp. Ref 1/W.

(2) A Plant Pathologist/Plant Breeder Scientific Officer (Band I) to evaluate *Allium cepa* germplasm for resistance to *Sclerotium cepivorum* (*Allium* white rot). Ref 2/W.

All appointments are initially for 1 year only but with good prospects for extension.

Qualifications: First or Upper second class honours degree, or equivalent in scientific or related subject for Band I, degree or equivalent with relevant post qualifying experience for Band II.

Salary in range of £6,711—£9,225. Non-contributory superannuation.

Apply for further details, quoting Ref number to Personnel Officer, Institute of Horticultural Research, Bradbourne House, East Malling, Maidstone, Kent ME19 6BJ.

The Institute of Horticultural Research is an equal opportunities employer. (2393)A

Lucille P. Markey Visiting Fellowships Program

Nominations are invited for postdoctoral fellowships under the above program. Up to four awards for a period of two years each will be made to outstanding candidates wishing to further their careers in biomedical science at a research institution in the U.S.A. Each awardee will receive a $25,000 stipend (plus $750 travel allowance) in the initial year, with a $3,000 increase in stipend for the second year. Candidates will be expected to specify the institutions at which they hope to work, the name of the preceptor under whose direction the work will proceed and obtain a commitment from the institution and preceptor that appropriate facilities for the research will be provided. The U.S. institution must have the appropriate tax exemption. Under the terms of the Lucille P. Markey Charitable Trust support is given for the study of fundamental biological processes rather than of specific disease entities and most awards will support work in fields such as molecular biology, developmental biology, neurobiology, immunobiology, immunology, genetics, virology and related areas of basic science.

Candidates *must* be nominated by a university or research institution in the U.K. and individual applications cannot be considered.

Further information and application forms can be obtained from:

Dr G. Stark
Imperial Cancer Research Fund
P.O. Box 123
Lincoln's Inn Fields
London WC2A 3PX

Closing date for completed applications: 1st June 1987.

(2252)E

POSTDOCTORAL FELLOWSHIP

A vacancy exists for a postdoctoral fellow interested in studying the regulation of immunity to tumour transplantation antigens and tumor histocompatibility antigens. Applicants should have a basic training in immunology. Starting salary will be $18,500 plus liberal fringe benefits. Subsidized Institute housing is available.

The Trudeau Institute is a private, not-for-profit, basic medical research institution located on the shore of Saranac Lake and near Lake Placid, NY.

Send curriculum vitae and names of three references to: Dr Robert J North, Director, Trudeau Institute, Inc., P.O. Box 59, Saranac Lake, NY 12983.

(NW578)E

UNIVERSITY OF OXFORD

MAGDALEN COLLEGE AND THE PHYSICAL CHEMISTRY LABORATORY

FIXED-TERM TUTORIAL FELLOWSHIP IN PHYSICAL CHEMISTRY

Magdalen College wishes to appoint a Tutorial Fellow in Physical Chemistry for a fixed period of three years, following the temporary suspension of university funding for a University Lectureship. The Fellow will teach up to 7 hours per week for the College and will undertake a programme of research in the Physical Chemistry Laboratory, where facilities will be made available. The Fellowship, which is non-renewable, is open to those under age 35 at the time of appointment. The stipend will be on an age-related scale rising to £10,973 at age 35. The Fellow will be provided with a study in the College, will be entitled to dinner and luncheon free of charge, and will be provided with free rooms in the College or a housing allowance of £1807 pa. Further particulars can be obtained from The ident's Secretary, Magdalen College, Oxford, OX1 4AU, to whom tions should be sent by April 24 1987.

(2391)E

UNIVERSITY OF SASKATCHEWAN

DRUG METABOLISM AND DRUG DISPOSITION RESEARCH GROUP

COLLEGE OF PHARMACY

POSTDOCTORAL FELLOWSHIPS

Applications are invited for two positions working as members of a large research group studying antipsychotic agents: (1) Synthesis and identification of metabolites; (2) Synthesis and metabolic application of deuterium labelled compounds.

The positions are tenable immediately and are for two years. Initial salary depends upon experience. Applications, including curriculum vitae and the names of three referees, should be sent to: **Dr K.K. Midha, College of Pharmacy, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, S7N 0WO (306) 966-6370).**

(NW651)E

THE UNIVERSITY OF AUCKLAND

New Zealand

POSTDOCTORAL FELLOWSHIP

DEPARTMENT OF ZOOLOGY

TOPIC: Biologically Active Metabolites of Marine Sponges, Chemistry, Pharmacology and Chemotaxonomy.

This Fellowship is available for a two year period, and must be taken up before 1 July 1988. Applicants should have a training in marine natural products, chemistry, and research level proficiency in GLC, HPLC and MNR techniques. There are opportunities for fieldwork in conjunction with marine biologists, and scuba diving ability would be an advantage. An annual stipend of NZ$30,500 will be paid, and airfare provided. Conditions of Appointment and Application Form are available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF; or the Assistant Registrar (Academic Appointments), University of Auckland, Private Bag, Auckland, New Zealand, to whom applications should be forwarded as soon as possible but no later than 8 May 1987.

(W3931)E

SENIOR RESEARCH FELLOWSHIP IN CARDIOVASCULAR RESEARCH

THE UNITED MEDICAL AND DENTAL SCHOOLS

The Rayne Institute,
St Thomas's Hospital

Applications are invited for th above post which is funded, to gether with generous runnin costs, for an initial 3 year perio The successful candidate, wh should have several years experi ence and a proven track record i cardica research, would be expec ted to establish and lead a tea within the Department. The n ture of the project would refl the interests of the Departmen and the candidate. Appointmen would be on the University's Re search and Analogous Staff Rang II/III scale according to age/quali fications and experience — th salary would be equivalent to senior lecturer or a senior positio on the lecturer scale.

Please apply with full CV an names and addresses of two referees as soon as possible to Professo David J Hearse, Director o Cardiovascular Research, Th Rayne Institute, St Thomas' Campus, UMDS, Lambeth Palac Road, London SE1 7EH.

(2403)E

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nature

the widest international selection of jobs
in science — EVERY WEEK

UNIVERSITY OF KENT AT CANTERBURY ■■■■

Biological Laboratory

Postdoctoral Molecular Biologist

Applications are invited for a 3-year AFRC-funded postdoctoral fellowship. The project under the supervision of Dr N Jenkins will investigate the hormonal control of protease gene expression in ovulation and infertility. Candidates must have experience in recombinant DNA techniques. Salary on the IA Scale for Research Staff.

Please apply by letter with a full curriculum vitae and giving the names and addresses of two referees to Mr I R Stone, Senior Assistant Registrar, Faculty of Natural Sciences, University of Kent, Canterbury, Kent CT2 7NZ quoting reference A87/43. (2374)E

NATIONAL INSTITUTE ON AGING

National Institutes of Health Public Health Service

The Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, invites applications for a Staff Fellowship position to study the factors that determine drug delivery to the brain, and their optimization. The successful candidate will interact with a group involved in: (1) the chemical modification of water-soluble drugs to increase their brain delivery and activity, brain directed prodrugs, (2) the chemical modification of centrally acting lipophilic drugs to extend their half-life, and (3) the design of therapeutic agents that are able to share cerebrovascular facilitated transport systems. The position is that of a Staff Fellow with a minimum salary of $20,000 per annum, duration 2 to 3 years. The position is located in Bethesda, Maryland.

Applications are invited from qualified individuals having a PhD, MD, or both, and experience in medicinal and analytical chemistry and interests in pharmacokinetics and the brain. Interested candidates should submit a curriculum vitae and a statement of their research interests to: **Stanley I. Rapoport, MD, Chief, Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, 9000 Rockville Pike, Building 10, Room 6C103, Bethesda, Maryland 20892.** *An Equal Opportunity Employer.* (NW646)E

THE ROYAL INSTITUTION DAVY FARADAY RESEARCH LABORATORY

Applications are invited from suitably qualified candidates for two Research Fellowships at postdoctoral level to pursue fundamental studies of porous solids and heterogeneous catalysts. The Davy Faraday Laboratory is well equipped for *in situ* X-ray and computer graphic work. Extensive use of centralized SERC facilities is envisaged as well as collaboration with British industry. Salary on scale £8,505 to £12,780 pa (under review) plus London allowance of £1,393. Starting date 1 May or as soon thereafter as possible.

Further particulars available from Professor J M Thomas, FRS, Director, Royal Institution, 21 Albemarle Street, London, W1X 4BS. (2387)E

STUDENTSHIPS

BRISTOL POLYTECHNIC

DEPARTMENT OF SCIENCE

RESEARCH STUDENTSHIP

Applications are invited for a SERC quota award studentship tenable for three years from students who have a first or upper second class honours degree. The project involves studying the mechanism of anti-viral activity of methyl xanthines in tissue culture cells.

Applications should include a curriculum vitae and names of two referees and should be sent to Dr D.G. Shaw, Microbiology Group, Department of Science, Bristol Polytechnic, Bristol, BS16 1QY, from whom more details may be obtained (0272 656261 ext 2482).

Applications should be received by 4 April 1987. (2378)F

THE UNIVERSITY OF MANCHESTER

Postgraduate Research Studentships

School of Biological Sciences

The School of Biological Sciences is a new initiative combining resources from the Science and Medical Faculties. Applications are invited for Postgraduate Research Studentships funded by the Medical Research Council, Ministry of Agriculture, Fisheries and Food, Natural Environment Research Council, Science and Engineering Research Council and the University of Manchester for research in the disciplines indicated below.

Department of Biochemistry & Molecular Biology

Research Groups:

- Biomolecular organisation and membrane technology
- Intermediary metabolism
- Matrix and tumour biology
- Molecular biology
- Protein and peptide engineering

Department of Cell and Structural Biology

Research Groups:

- Animal and human reproduction, development and growth
- Chemical morphology
- Contractile and excitable tissues
- Experimental neurology
- Immunology
- Microbiology
- Plant sciences and cytogenetics

Department of Environmental Biology

Research Groups:

- Environmental physiology
- Pollution biology and ecosystem management
- Population and evolutionary biology (and, in addition, M.Ce. course in Pollution and Environmental Control)

Department of Physiological Sciences

Research Groups:

- Chronobiology
- Epithelial transport and secretion
- Neurosciences
- Renal physiology and endocrinology
- Reproduction
- Smooth muscle
- Therapeutic drug monitoring

Candidates for M.Sc./Ph.D. courses in these Departments must hold, or expect to obtain, a first or 2(i) Honours Degree in an appropriate subject. For further information and application forms write (quoting reference RS/AD and stating preferred research group) to Professor A.P.J. Trinci, *School of Biological Sciences, Williamson Building, the University, Manchester M13 9PL.* (2396)F

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nature

Still first with the best in science

UNIVERSITY OF NOTTINGHAM

CANCER RESEARCH CAMPAIGN LABORATORIES

PhD STUDENTSHIPS

Applications are invited from candidates who have or expect to have a first or upper second class honours degree in biochemistry, immunology, cell biology or a related subject.

Successful applicants will join a team of research scientists investigating, designing and developing monoclonal antibody-drug conjugates for cancer treatment. The areas of research include the design of drug-antibody conjugates, assessment of their cytotoxic properties and control of immunological responses.

Please send curriculum vitae and the names and addresses of two academic referees to: **The Secretary, Cancer Research Campaign Laboratories, University of Nottingham, Nottingham, NG7 2RD.**

(2372)F

ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND

ANATOMICAL SOCIETY GRADUATE STUDENTSHIPS

Applications are invited for Studentships tenable in the Anatomical Sciences for a period up to three years. Nominees would be expected to work for a higher degree by research. Candidates must be graduates of British or Irish Universities and must have arranged sponsorship with a Department of Anatomical Sciences in the UK or Ireland. The stipend will be commensurate with basic Research Council rates.

ANATOMICAL SOCIETY SENIOR VISITING FELLOWSHIP

Applications from Overseas Scientists are invited for one Fellowship tenable for a period of less than a year in a Department of Anatomical Sciences in the UK or Ireland. Applicants should be of postdoctoral or comparable status and must have arranged sponsorship in the Department in which they intend to work. Some assistance towards travel and subsistence will be provided.

Further particulars of the Appointments should be obtained from the Honorary Secretary, Professor J A Gosling, Department of Anatomy, Stopford Building, University of Manchester, Oxford Road, Manchester, M13 9PT, to whom applications should be sent by July 15th Applications for studentships must be accompanied by sealed of support from two referees. (2122)F

UNIVERSITY OF DUNDEE

DEPARTMENT OF BIOCHEMISTRY

PhD STUDENTSHIPS

Applications are invited for one or more studentships to be supervised by Dr Grahame Hardie in the following areas:

(1) an earmarked SERC Biotechnology Directorate studentship is available in the area of plant biotechnology, to be carried out in collaboration with Unilever Research. The project will involve purification, sequencing and cloning of enzymes of fatty acid biosynthesis in oil-seed rape.

(2) a Departmental MRC or SERC studentship may also be available in the area of the structure, and regulation by phosphorylation, of enzymes of mammalian fatty acid and cholesterol synthesis.

Candidates should have British resident status and should hold, or expect to hold by June 1987, at least an upper second class Honours degree in Biochemistry or a related discipline. Send a curriculum vitae (including telephone no. if possible) and the names and addresses of two referees, to **Dr D.G. Hardie, Department of Biochemistry, The University, Dundee, DD1 4HN,** as soon as possible (telephone 0382-23181 ext 4253 for further details). (2381)F

LUDWIG INSTITUTE FOR CANCER RESEARCH

Cambridge Branch

PhD STUDENTSHIP

Applications are invited for a PhD studentship available for three years from October 1987 to work on oncogenes and anti-oncogenes in human cancer.

Applicants should have a first or upper second class honours degree in a biological or related subject.

Applications including up-to-date c.v. together with the names of two referees should be sent to: **The Administrator, Ludwig Institute for Cancer Research, MRC Centre, Hills Road, Cambridge CB2 2QH.**

(2330)F

PhD Studentship

Applications are invited for a PhD studentship to work on mechanisms of action of anthracycline anti-cancer drug-loaded protein micro-spheres. The successful candidate will be a member of the anti-cancer drug development group based in the ICRF Medical Oncology Unit, Western General Hospital, Edinburgh, but much of the work will be in the department of Pharmacy, University of Strathclyde. Applicants should possess or expect to obtain a good honours degree in pharmacology, pharmacy or biology.

Enquiries to: **Dr Cummings, ICRF Medical Oncology Unit, Edinburgh (031 332 2471).** Full curriculum vitae and names and addresses of three referees should be sent to the **Personnel Officer, Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX** quoting ref: 80/87R. (2409)F

IMPERIAL

CANCER RESEARCH FUND

SEMINARS & SYMPOSIA

LEUKAEMIA RESEARCH FUND

International Research Symposium on

"CHROMOSOMES AND GENES IN LEUKAEMIA"

at the Royal College of Physicians, London NW1

on MONDAY 27th APRIL 1987

Registration fee (including lunch): £15

For programme details and registration form apply to:

LEUKAEMIA RESEARCH FUND, 43 Great Ormond Street, London WC1N 3JJ (tel: 01 405 0101)

(1796)M

SEMINARS & SYMPOSIA continued

Sandoz Institute for Medical Research

3rd Annual Symposium

Ion channels and their regulation

to be held at The Royal Society
6 Carlton House Terrace, London

FRIDAY 15 MAY 1987

Commence 9 a.m.

Speakers and Provisional Titles

Dr H Reuter, Berne,	"Ion Channels – An overview"
Dr W Stühmer, Göttingen,	"cDNA-derived Sodium Channels"
Dr R Miller, Chicago,	"Receptor-induced Modulation of Calcium Channels"
Dr S Cull-Candy, London,	"Amino Acid Gated Channels"
Dr J Lemos, Shrewsbury,	"Ion Channel Reconstitution in Lipid Bilayers"
Dr R Huganir, New York,	"Role of Phosphorylation in Ion Channel Activity"
Dr R Irvine, Cambridge,	"Hormone-Receptor Coupling"

Admission to the Symposium will be limited to registrants. No charge will be made for registration, which will include a buffet lunch (number limited).

Registration forms obtainable from: Mrs M C Stuart, Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN, to be returned by Friday 24 April 1987.

SANDOZ

(2364)M

—SECOND ANNOUNCEMENT–CALL FOR ABSTRACTS—

7th INTERNATIONAL SYMPOSIUM ON AFFINITY CHROMATOGRAPHY AND INTERFACIAL MACROMOLECULAR INTERACTIONS

TO BE HELD AT OBERAMMERGAU, WEST GERMANY ON AUGUST 17-21, 1987

Scientific Sponsors: Ludwig-Maximilians-Universität Munchen, Gesellschaft für Biologische Chemie (GBCh), International Interest Group in Biorecognition Technology (IIGBT).

Organizers: H.P. Jennissen (Munich), Chairman and W. Müller (Darmstadt).

Scientific Program:

1. FUNDAMENTALS OF MOLECULAR RECOGNITION
2. POLYMER AND PROTEIN ADSORPTION
3. NEW DEVELOPMENTS IN AFFINITY SEPARATIONS
4. ANALYTICAL AND CLINICAL APPLICATIONS OF AFFINITY TECHNIQUES
5. INDUSTRIAL AND BIOTECHNOLOGICAL APPLICATIONS OF AFFINITY TECHNIQUES

The Symposium will have the following format:
MAIN SESSIONS WITH INVITED SPEAKERS AND POSTER SESSIONS

Speakers will include:
Brash, J.L. (Hamilton); Brooks, D.E. (Vancouver); Chaiken, I.M. (Bethesda); Crothers, D. (New Haven); Fleer, G.J. (Wageningen); Hjertén, S. (Uppsala); Horvath, C. (New Haven); Huber, R. (Martinsried); Janson, J.–C. (Uppsala); Karube, I. (Yokohama); Lowe, C.R. (Cambridge); Mosbach, K. (Lund); Porath, J. (Uppsala); Regnier, F. (Lafayette); Shaltiel, S. (Rehovot); Ugelstad, J. (Trondheim); Uhlén, M. (Stockholm); Wilchek, M. (Rehovot).

Deadline for the submission of abstracts for poster sessions: April 15, 1987.

The abstracts will be published in the journal "Biological Chemistry Hoppe Seyler" at the time of the Symposium.

There will be a COMMERCIAL EXHIBITION: "Affinity 87".

For Correspondence and Registrations:
Deutsches Reisebüro GmbH, DER Congress, Att. Mrs B. Schönfeldt, P.O. Box 100701, D-6000 Frankfurt 1, West Germany; Telephone: 069-1566 380, Telex: 41529270 dr d, Telefax: 069-1566-573. (W3928)M

WORKSHOPS

CNR Special Project IPRA
EMBO

WORKSHOP ON PLANT DEVELOPMENT

It will be organized by M. Terzi and Z.R. Sung at Vallombrosa, near Florence, on July 20-23, 1987

Speakers will include:
M. Christianson (San Francisco), D. Dudits (Szeged), R. Goldberg (San Francisco), S. Hake (Berkeley), A. van Kammen (Wageningen), D. Kapian (Berkeley), A. Komamine (Sendai), H. Lörz (Cologne), F. LoSchiavo (Naples), M. van Montagu (Ghent), V. Nuti (Pisa), F. Salamini (Cologne), T. Thomas (College Station), S. de Vries (Wageningen).

The following topics will be discussed:

1. Mechanisms of morphogenesis
2. Induction of developmental programme
3. Developmentally regulated genes
4. Developmental mutants and insertional mutagenesis
5. Functional analysis by reverse genetics

Applications, arriving not later than May 10, should include a c.v. and a brief description of research interests. They should be addressed to **Professor Mario Terzi, Istituto di Mutagenesi e Differenziamento, CNR, Via Svezia, 10. I-56100 Pisa, Italy.**

A small number of travel grants will be available to participants without alternative sources of support.

(W3925)V

CONFERENCES & COURSES

IOB **Institute of Biology**
Incorporated by Royal Charter

THE LESSONS OF CHERNOBYL

EFFECTS ON MAN AND HIS ENVIRONMENT

Dr Robert Gale (Los Angeles) and speakers from USSR and Europe

London, 1000-1700, Saturday, 11 April

Admission by ticket only

Registration £25 (£20 Institute Members)

Bookings and enquiries to:
General Secretary, Institute of Biology
20 Queensberry Place, London SW7 2DZ

(2369)C

33rd SCOTTISH UNIVERSITIES SUMMER SCHOOL IN PHYSICS
ST. ANDREWS
14th–25th SEPTEMBER 1987

ASTROPHYSICAL & LABORATORY PLASMA SPECTROSCOPY (NATO ASI)

Further information and application forms from: **Dr. D.E. KIDD, Dept. of Physics and Applied Physics, University of Strathclyde, 107 Rottenrow, Glasgow G4 0NG, Scotland.**

(Closing date for applications 1st May 1987) (2361)C

continued on page 2

THE GLOBAL IMPACT OF
A.I.D.S.
8-10 MARCH 1988.

Organised by London School of Hygiene and Tropical Medicine in association with World Health Organisation, Commonwealth Secretariat and the U.K. Department of Health and Social Security.

Considerable multidisciplinary research resources have been directed towards the problems of A.I.D.S., so far mainly those of diagnosis, treatment and vaccine development. There is a growing understanding of the viruses and the pathogenesis of the disease.

By 1988 it will be possible to look at the initial results of current A.I.D.S. research in a much wider range of disciplines and to begin to see the possible long term implications.

These will be apparent for primary health care and health care systems, community health, personal and national finances, employment and industry, international travel, international relations and aid for the developing world.

This meeting will concentrate on an in-depth analysis and evaluation of the demographic, economic, psychological and educational aspects of the A.I.D.S. problem, based on the results of current research. The conference will have key note addresses, poster sessions and a main theme based on the results of current work.

Cost of the conference will be £250 + V.A.T.

For details and bookings contact:
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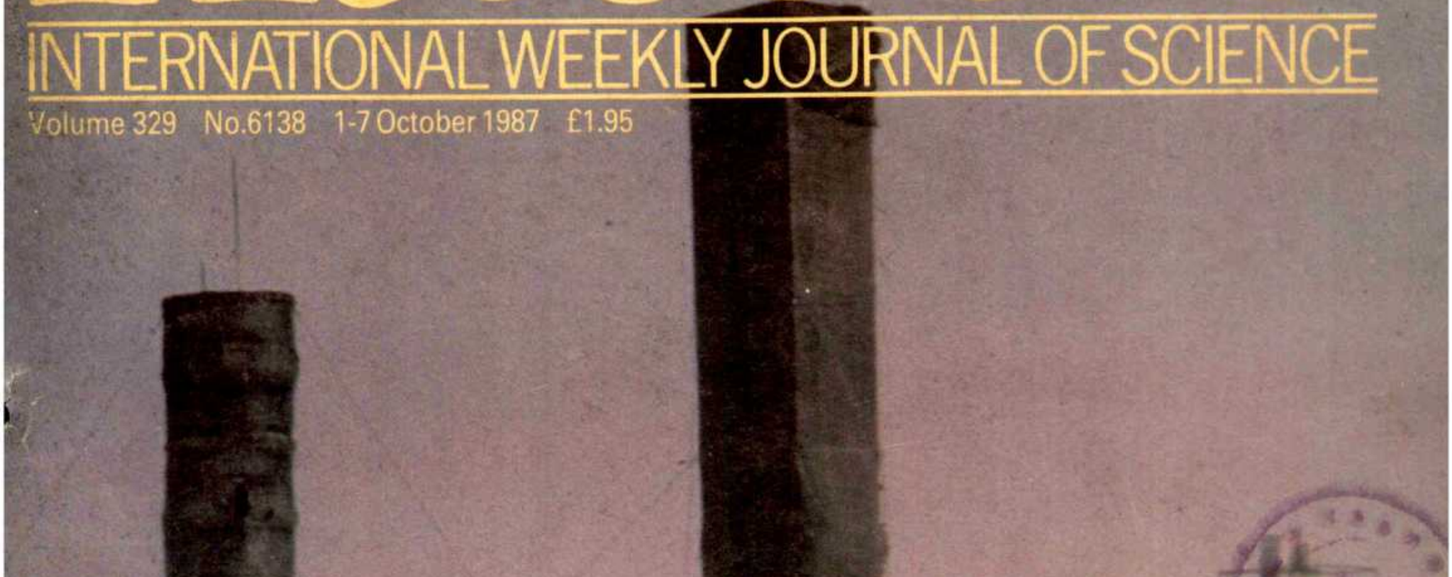

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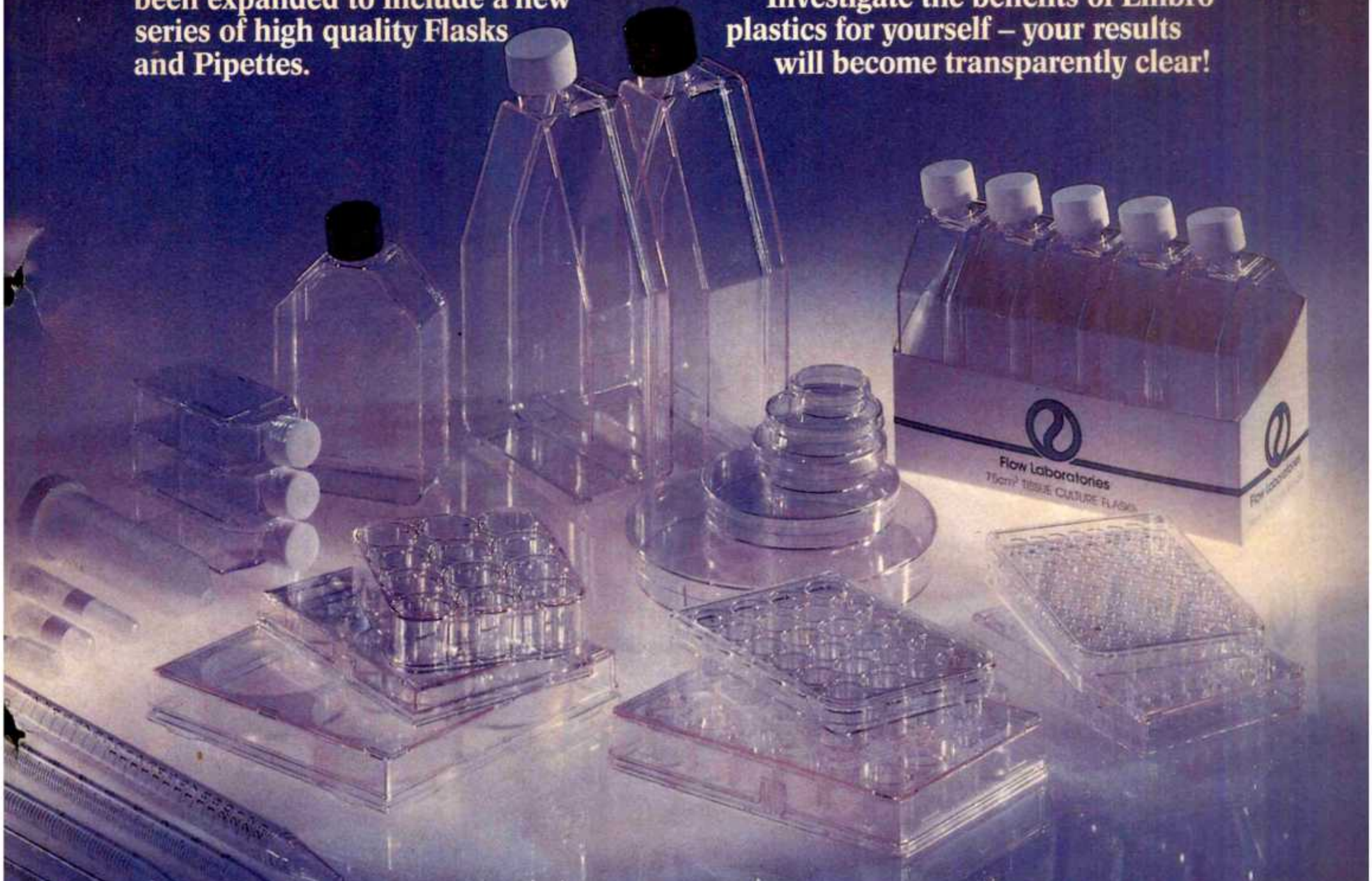

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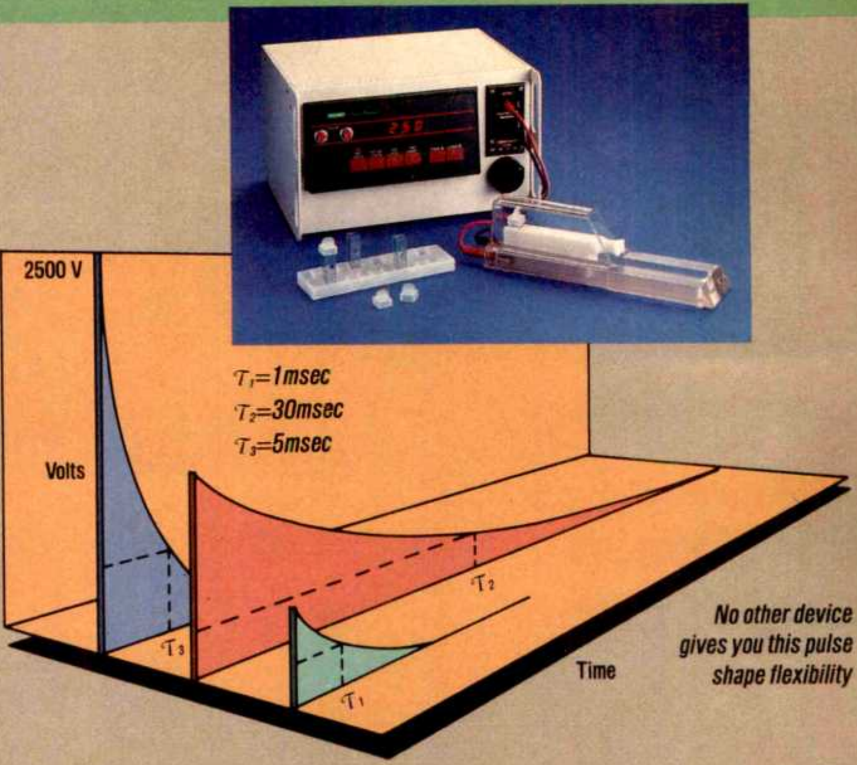

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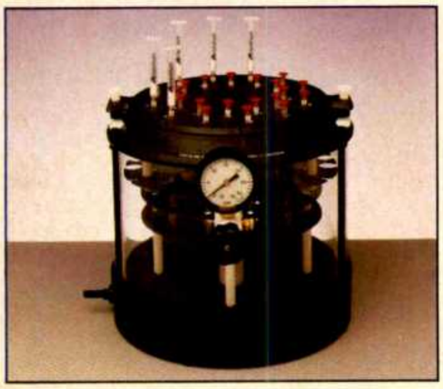

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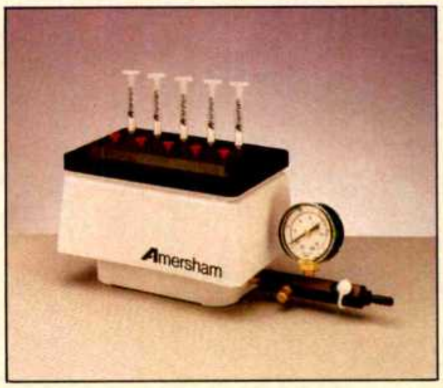

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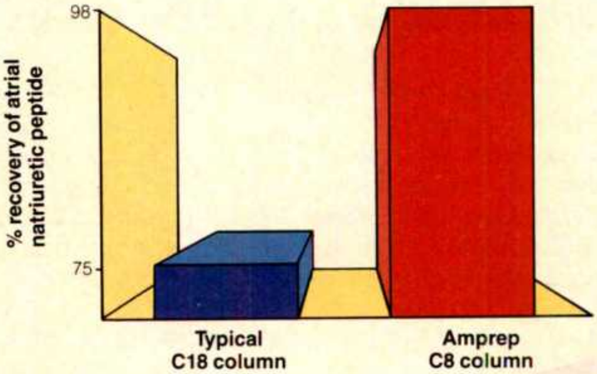

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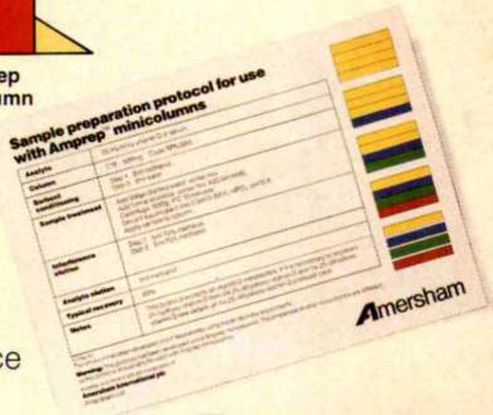

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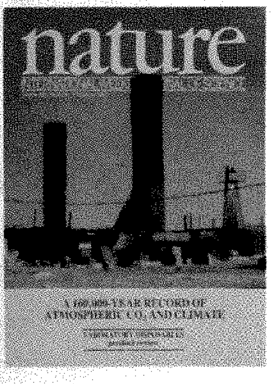

nature

1 October 1987

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Vostok, the Soviet station in East Antarctica. The three turrets mark the sites of several attempts, since 1972, to penetrate to significant depths. Results from the most successful, at a depth of 2.2 km, are reported on pages 403–418 and discussed on pages 387 and 389. They provide a record of atmospheric composition and of climate stretching over two ice-ages.

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Cytotoxic T cells

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A real globin?

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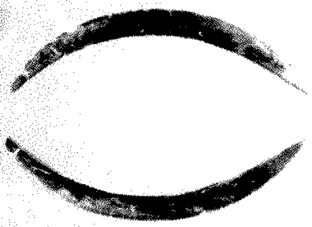
Polish boomerang



The oldest boomerang in the world — made from a mammoth tusk — has been discovered in a cave in Poland, pages 436 and 388.

Neuron model

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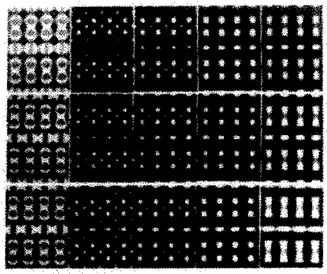

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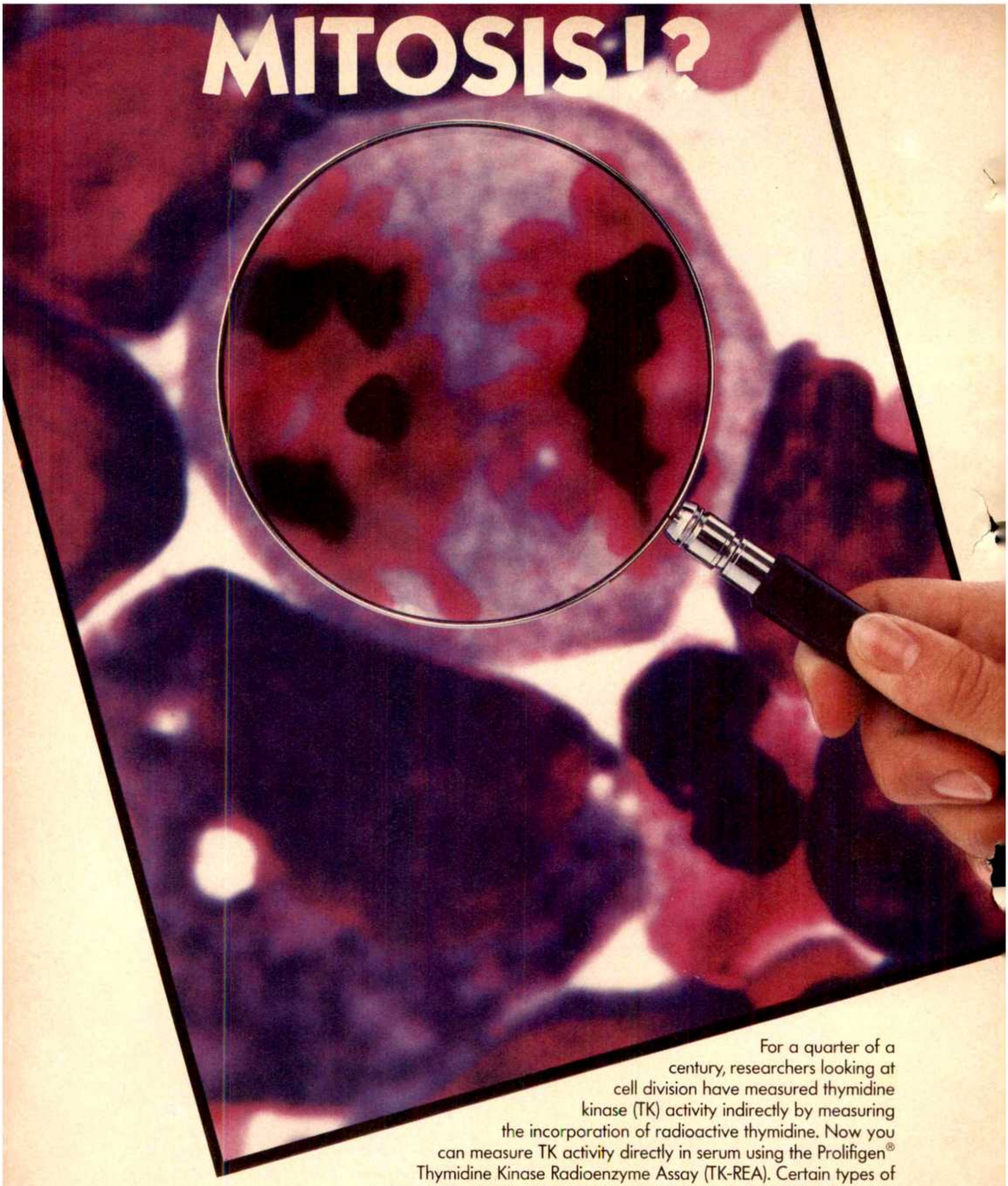

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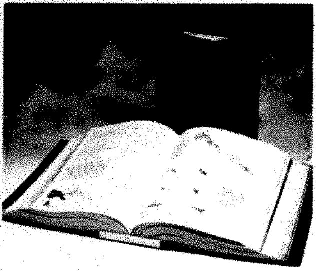

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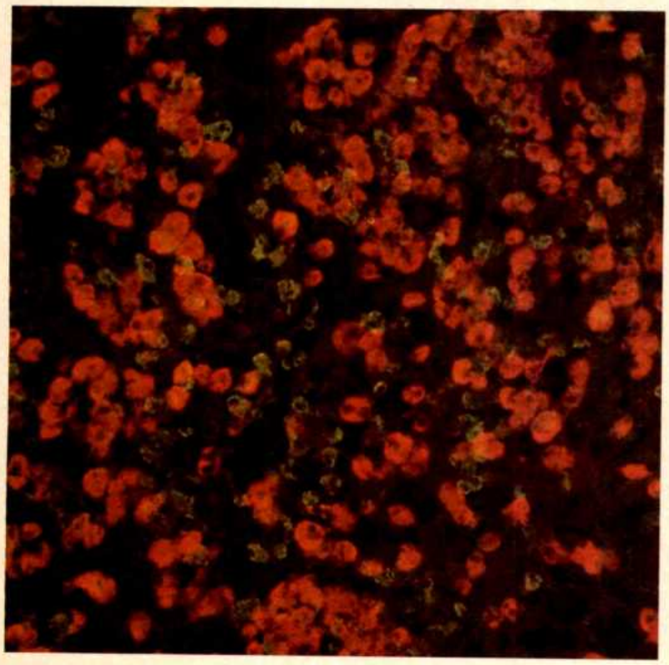

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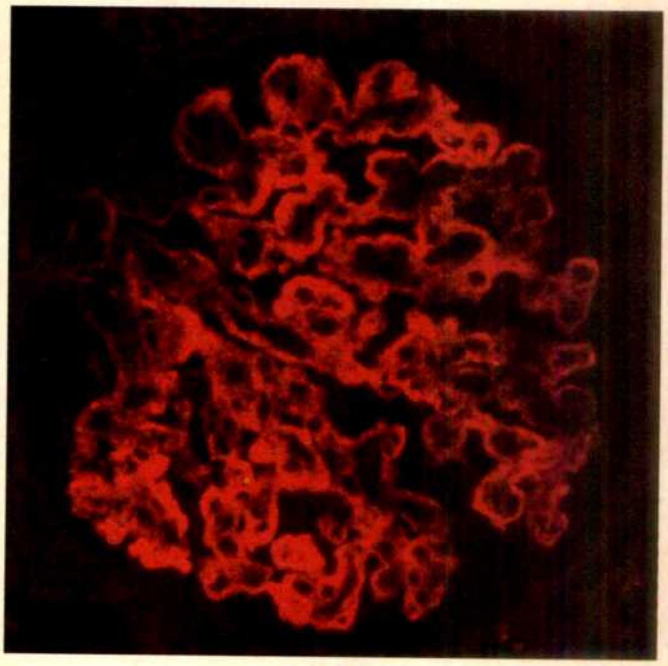

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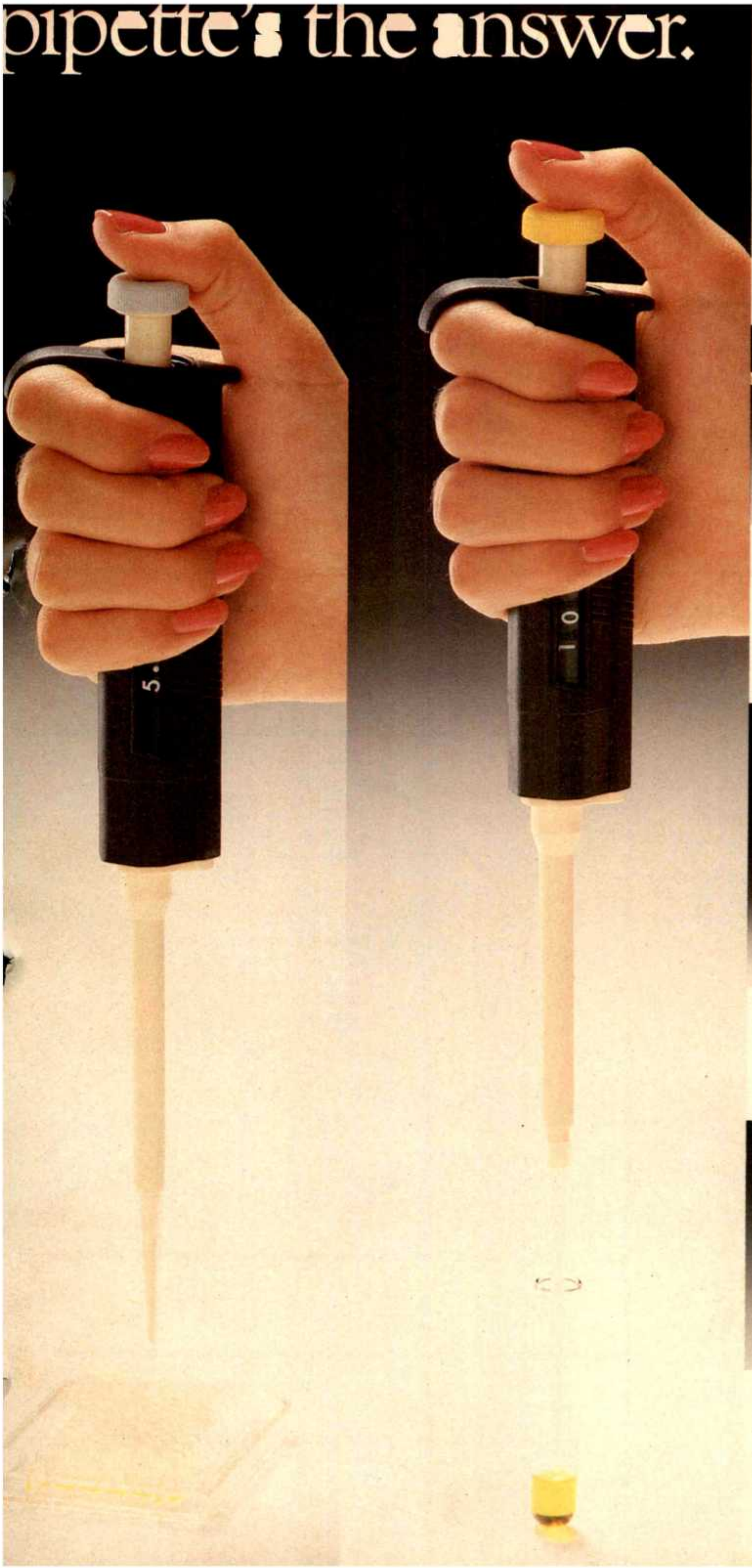

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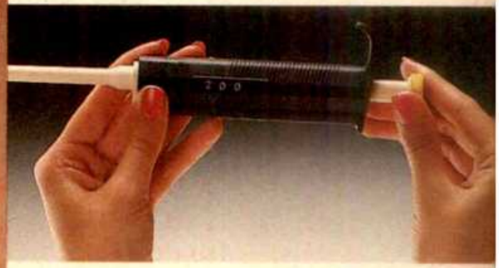

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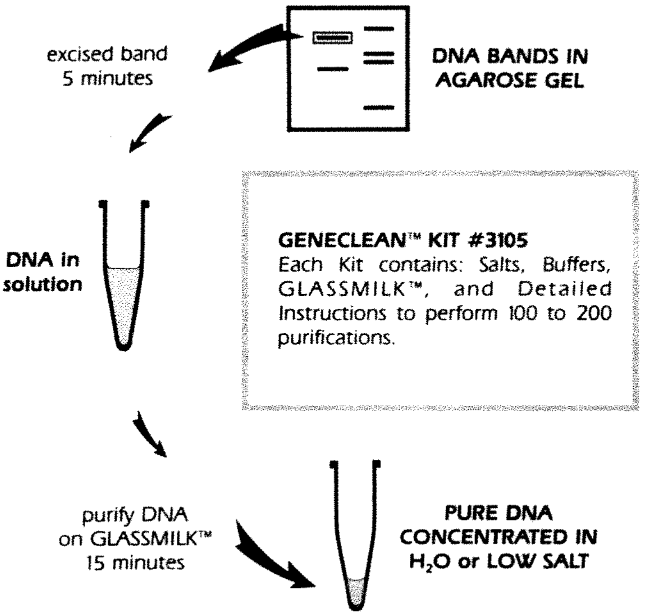
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What Molecular Biology Means for the Medical Sciences

VACCINE DEVELOPMENT TODAY AND TOMORROW

Hepatitis, the First Success

Pablo Valenzuela

Chiron

The Design of Subunit Vaccines for Production in Yeast Cells

Arthur Levinson

Genentech

The Design and Production of Subunit Vaccines in Mammalian Cells

Malaria, the Goal is in Sight

Victor Nussenzweig

New York University Medical Center

How Close are We to a Sporozoite Vaccine, and How Effective is it Likely to be?

AIDS, the Challenge on the Horizon

Flossie Wong-Staal

U.S. National Institutes of Health

The Molecular Biology of Human Immunodeficiency Virus

Myron Essex

Harvard University School of Public Health

The Natural Biology of AIDS: Lessons for a Potential Vaccine

● TUESDAY, OCT. 6

BIOLOGICAL RESPONSE MODIFIERS: PROMISE AND REALITY

George Poste

Smith, Kline & French Research Laboratories

The Challenges and Opportunities of Biological Response Modifier Therapy

Bart Sefton

Salk Institute

The Role of Receptor Signaling in Cellular Responses to Growth Factors

Kendall Smith

Dartmouth Medical School

The Structure-Activity Relationships Between Interleukin-2 and its Receptor

Y.H. Tan

Institute of Molecular and Cellular Biology, National University of Singapore

Intracellular Signaling in the Interferon System

Ken-ichi Arai

DNAX Institute of Molecular and Cellular Biology

The Molecular Biology of T-Cell-Derived Lymphokines

David Goeddel

Genentech

Will Tumor Necrosis Factor and Related Molecules Become Effective Cancer Therapeutics?

● WEDNESDAY, OCT. 7

ACCESSING THE HUMAN GENOME

Leroy Hood

California Institute of Technology

How Far are We from Sequencing the Human Genome, and What Will We Learn in the Process?

Anthony Monaco

The Boston Children's Hospital

The Isolation and Characterization of the Duchenne Muscular Dystrophy Gene, and its Implications for Treatment

Henry Erlich

Cetus

The Application of DNA Probe Technologies to the Identification of Genetic Disorders

Jan Breslow

The Rockefeller University

The Characterization of Human Apolipoprotein Genes, and Their Contribution to Atherosclerosis

Marcello Siniscalco

Memorial Sloan-Kettering Cancer Center

Chromosome Fragility, Genetic Recombination, and Human Disease

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nature

NATURE VOL. 329 1 OCTOBER 1987

Getting to grips with fraud

Dealing with scientific fraud is likely to be an unpleasant process for accused and accuser alike. The only solution lies in well conceived methods of procedure.

UNTIL recently there would seem to have been little need for discussion of the dangers of the fraudulent reporting of data to the scientific enterprise. There was always the odd historical case of fraud and every generation seemed to bring forth at least a handful of people who were determined to identify finally the perpetrators of the Piltdown Man hoax. But that was a pastime conducted in a similar obsessive spirit to the hunt for the identity of the "dark lady" of Shakespeare's sonnets and seemed to have little relevance to the daily practice of the scientific profession.

In retrospect, 1980 proved a turning point with allegations of falsification of data made in four major cases. The pace of discovery of new cases of fraud, malpractice and plagiarism has not slackened subsequently. Since 1980, such allegations have been made publicly in at least twenty-five cases and cleared up privately in a further seven cases.

These are the figures, not necessarily comprehensive, that obtained at a workshop on scientific fraud and misconduct sponsored jointly by the American Association for the Advancement of Science and the American Bar Association last week. The figures are not by themselves particularly worrying. Even though more than a hundred papers tainted by fraud may have been produced, that number is still insignificant compared to the world output of scientific literature. But there remain several issues to worry about.

How are scientific frauds detected? Conventional wisdom has it that science is self-correcting: experiments are repeatable and a fraudulent or incorrect result will inevitably be detected in a later repetition. But in much of the biological sciences, where a great measure of skill and experience is needed to master new techniques, things are not so simple. And in the medical sciences, where human subjects are involved, experimental opportunities may have to be taken as they arise, and cannot be totally controlled or perfectly repeated. To say these things does not call into question the basic principle that experiments are repeatable. But it does mean that the first thought of someone who fails to replicate a result is not likely to be that the result is fraudulent. A more charitable explanation is likely to be sought in minor differences in experimental conditions.

For these reasons it appears that frauds have rather rarely been discovered through the replication of experiments by totally independent groups. The discovery of fraud is more usually a personal affair: a co-worker's suspicions are aroused, and then confirmed by events extraneous to the published record — for example, that a reagent essential to the experiment had not been ordered at that time, or even that the number of Petri dishes used in an experiment was greater than the number the laboratory had in stock.

The personal nature of the discovery of fraud inevitably makes it a painful business. It is not like having been a chance witness at a bank robbery. No one is going to emerge as a hero from an investigation which is likely to blacken not just the name of the individual scientist but also his or her institute and the scientific enterprise itself. But that should not mean that the person who exposes a fraud is penalized for his or her courage.

Unfortunately, the experiences of "whistle-blowers", personally related at the workshop, makes clear that the price of exposing fraud is high. Action by institutions, which clearly saw their own reputations at stake, was often taken grudgingly and after years of delay. The motives of the accuser were frequently questioned: did jealousy or personal animosity play a part?

There seems no escaping the conclusion that for both accuser and accused to have full protection of their good reputations, which is ultimately all that a scientist has to assure continued employment, universities need effective processes for dealing with allegations of fraud and dispensing justice rapidly. Some universities — mainly those who have been through the protracted trauma of a fraud case — have already made a start but their experiences show that it is not easy. The skills for investigating fraud, different from those necessary for research, are hard to obtain. And universities have genuine conflicts of interest between dispensing justice and protecting their reputation that may make systematic policies and procedures unpalatable.

There is little hope that universities transfer their responsibility to investigate fraud to other bodies (except when the misuse of public funds makes it necessary to involve the granting agencies). Scientific societies may help to set guidelines of proper practice but they are not in a position to deal with individual cases. Nor can a great deal of help be expected from scientific journals. Although journals may choose to publish letters that point out inconsistencies in, or failures to replicate, previously published work, they would find themselves in a difficult legal position if they began to make accusations about their own authors.

Could journals do more to ensure that fraudulent work never appears in print? "Why ever was the paper accepted" is a natural enough reaction when a case of fraud is uncovered. But evidence of fraud is not necessarily to be found in the the published account. And while conscientiousness can be expected of referees, the total scepticism needed to suspect that an author's every statement may be false, surely cannot. Even scepticism may be insufficient for fraudulent work is often correct in its conclusions. That need cause no surprise. People who commit fraud are only very rarely stupid enough to falsify scientific breakthroughs. More characteristic is a pedestrian piece of work with a wholly expected result. But that that is so forces a hard question: How different is fraud from shoddy practice?

If the timid manufacture of data to conform to already probable hypotheses is the most common form of fraud is it one end of a spectrum of shoddy practice that includes the selection of data, the filling in of inadequate or control data through phentom experiments, and the use of guest co-authors to add credibility to mediocre work? The more comforting view is that frauds are committed by a few highly deviant individuals whose activities have nothing to do with the normal scientist. Between these two hypotheses — that fraud represents either the tip of an iceberg or a few bad apples — there are not data to make a judgement, although practising scientists clearly each have their own view. But a corollorary of the first hypothesis is that there are rewards for the mediocre work that no one will ever read carefully. That leads to a much larger question: has the scientific enterprise come to stress quantity to such an extent that quality has begun to suffer? □

Japanese industry dreams of superconductor future

■ Parliament lobbied for more cash

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Tokyo

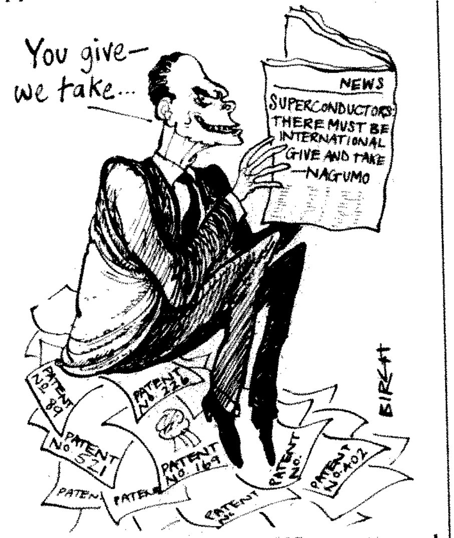
How does Japan foresee the development of high-temperature superconductors? A recent meeting between scientists and members of the House of Councillors provides some clues.

At the end of last month, four leading superconductor researchers briefed members of the special committee for science and technology of the upper house of the Diet. Ruichi Nakagawa, Director of the Science and Technology Agency's National Research Institute for Metals, began the 3½-hour session with a simple explanation of superconductivity. To convey the importance of zero resistance to the Diet members he pointed out that thermal losses due to resistance in Japan's national electricity grid equal half the electricity consumption of Tokyo, or ¥600,000 million a year (more than $4,000 million). Professor Kazuoki Fueki of Tokyo University explained resistivity at the atomic level and the notoriously complex 'BSC theory' of superconductivity, while Michihiko Nagumo of Shin Nippon Steel and Shigeru Hayakawa of Matsushita Electric Co. pointed out the potential practical applications.

The four researchers were unanimous in stressing the need for more basic research into the properties of the new materials and a theory to explain high-T_c superconductivity. They also called for international collaboration in research. The barriers to development of practical applications, such as the low critical current densities of the bulk oxides, their fragility and sensitivity to oxygen and the difficulties of processing them into wires, were emphasized. But this did not stop Hayakawa "dreaming" about the future. He foresees applications including gigantic liquid-crystal displays networked with superconducting wire, high-density memory devices such as centimetre-long video tapes with superconducting memory loops, supersensitive Josephson junction devices capable of monitoring the magnetic fields of the brain and heart, high-speed Josephson computers, and high-definition satellite television.

Professor Fueki complained that lack of money is the major problem besetting basic research in the universities, a view echoed by Nakagawa, who pointed out that high-field magnets to measure magnetic-field properties of the new materials cost several hundred million yen (several million dollars) each. And the Science and Technology Agency has recently applied for over ¥1,000 million to build three high-field magnets at Nakagawa's institute next year (see *Nature* **329**, 93; 1987).

On the issue of patents, there were fears that the United States may rush to develop technology and shut out Japan. Nagumo argued that the new superconductors could lead to a new industrial revolution, and that patents should not be allowed to block the way; instead there should be international "give-and-take". Hayakawa called for "open patents" that allow licensing by anyone, and he suggested that rapid development of consumer products could forestall the classification of superconductor applications as military secrets. But Sumitomo Electric Co., a cable manufacturer, is taking no chances. The company has already filed over 600 patent applications for the new superconductors in the hope of blocking US patents and arranging cross-licensing agreements.



Japan also fears that it may prove difficult to secure adequate supplies of the raw materials to make high-T_c superconductors. In reply to a question from house member Masao Goto, Nagumo said that Japan will need to import several million tons per year of rare earth elements such as yttrium, as opposed to the few thousand tons currently imported.

The meeting closed with a question the experts could not answer. Hiramatsu Deguchi asked about the effects of magnetic fields on people's health. Both Fueki and Nakagawa admitted that the medical effects of magnetic fields are unknown, and they called for more research into this matter.

David Swinbanks

Academy pushes basics too

Washington

US government agencies are in the midst of a debate over how best to pursue the potential of the newly discovered class of superconducting materials. The National Academy of Sciences has now weighed in with its perspective*, urging the government not to ignore the science of superconductors while it pursues their technological promise. The Academy report also cautions that interest in superconductors could place demands on the scientific and technical manpower pool beyond its capacity to respond.

The report, produced by a panel of the committee on science, engineering and public policy, says a national programme to exploit the new materials should focus on a better understanding of their essential properties, as well as the mechanism repsonsible for those properties. In addition to searching for new superconducting materials, the report suggests starting the process of creating prototype devices that might incorporate the new materials. For the next fiscal year, the panel reckons that $100 million will provide "a good beginning" in exploring superconducting compounds and their potential uses.

The rallying cry of improving economic competitiveness has frequently been sounded to justify research on superconductivity. Both Congress and the Reagan administration are anxious that research advances be translated into commercial products in a timely fashion. But the Academy report points out that the world superconductivity market is relatively small, although products made with superconductors — especially medical imaging devices — represent a much larger market. Given the engineering problems presented by the ceramic superconducting materials, the panel expects it will take a decade of precommercial exploration to establish the range of potential applications. Although the panel is unanimous that the new materials are bound to result in new economic growth, it will take room-temperature superconductivity to bring about a revolution.

The attraction of working with superconductors, both because of the intellectual excitement they have generated and the financial backing they are likely to attract, may syphon off engineers from other research areas where they cannot easily be replaced. The report urges that attention be paid to the educational demands that must be met if adequate numbers of suitably trained people are to continue to enter the field. **Joseph Palca**

**High-Temperature Superconductivity, Committee on Science, Engineering, and Public Policy, National Academy of Sciences, Washington, D.C.*

Pakistani satellites

PAKISTAN is close to making a decision on plans for the launch of a pair of direct television broadcast satellites. According to the chairman of Pakistan's Space and Upper Atmosphere Research Commission (SUPARCO), Salim Mehamood, the Parliament will soon have to debate the funding share of the various agencies that will use the two "Paksat" satellites.

Pakistan has been stimulated by Indian successes with its SLV-3 rocket, which Pakistan claims could be used as an intermediate-range ballistic missile, to give more weight to space development. A ten-year plan stressing the need for indigenous launch capacity has been released and earlier this year a new facility for the production of solid fuel was inaugurated. At present, Pakistan's capabilities encompass only the production and launch of sounding rockets from its Sonamiani Beach base near Karachi. Paksat would be launched abroad.

Space development could well be hastened by cooperation with other Islamic countries. Serious efforts are being made towards the creation of an Islamic Space Institute with Bangladesh, Egypt, Saudi Arabia, Indonesia, Iran and Turkey as partners. R.R.

Collider sites

OF the 43 sites proposed for the Superconducting Super Collider (SSC), 36 have survived internal screening by the US Department of Energy (DoE), and have been passed on to the National Academies of Sciences and Engineering for more detailed assessment and reduction to a shortlist, to be announced next January (*Nature* 329, 92; 1987).

The only rejected site with official state backing was New York's proposal to build the SSC partly in Canada. Three New York locations wholly within the United States remain in contention. Bids were also disqualified if they did not fully demonstrate that the site could physically accommodate the SSC layout, had adequate electricity or water supply, would come free of cost, or was environmentally acceptable. Three of the unsuccessful bids were in Texas, but the state still has two official sites and two backed by county governments, as well as an interest in a site in New Mexico. D.L.

British space lobby

BRITISH industry is continuing to lobby the government for greater investment in space research. British Aerospace has written to 135 members of parliament and peers of the House of Lords pointing out the benefits to the economy of an expanded space programme. In particular, the need for Britain to at least retain its current position in the European Space Agency is emphasized. S.H.

Britain's genetic manipulation regulations to be extended

London

VOLUNTARY schemes for notification of the deliberate release or industrial use of genetically manipulated organisms in Britain are likely to be replaced by compulsory notification. Any failure to notify the Health and Safety Executive (HSE), whose commission proposed the changes last week, would become a legal offence.

The Health and Safety Commission, which put forward the changes after consultation with its Advisory Committee on Genetic Manipulation, says its main objectives are to ensure the proper risk-assessment of planned release and industrial use of altered organisms, and effective inspection of the establishments concerned.

But the changes are also designed to remove the anomaly that notification of the genetic manipulation of organisms is already compulsory, whereas the employment of the organisms, which may well carry a greater risk, is not. The changes are expected to anticipate the recommendations of the Royal Commission on Environmental Pollution that is due to report on deliberate release experiments within the next few months.

The HSE also hopes to make compulsory two other aspects of genetic manipulation that are currently voluntary. One would make it essential for anyone notifying the HSE of a genetic manipulation experiment to assess the risk of the experiment by a method approved by the HSE. The other would make it compulsory for any establishment that carries out genetic manipulation to set up a safety committee.

Other changes proposed in last week's consultative paper are designed to close two loopholes in existing regulations. No longer would it be possible, in theory, to argue that the direct incorporation (by, for example, microinjection) of foreign DNA into cells is not subject to regulation. And deliberate release from offshore installations (an oil rig, for example) would unambiguously be covered by the new regulations, although strangely they would not apply to ships in territorial waters or aircraft flying over them.

In one respect the new regulations would somewhat lighten the bureaucratic load on British genetic manipulators. Those involved in work that is categorized as low risk will need only to give numbers rather than details of their experiments in the annual retrospective notification they have to file. **Peter Newmark**

■ The UK Department of Trade and Industry is hoping to interest companies in jointly funded experiments to assess the risk of deliberately releasing genetically manipulated plants or microbes. If experiments of sufficient interest to a number of companies can be identified, the department's Biotechnology Unit is prepared to pay half of their cost.

Roy Dietz, head of the unit, anticipates that such experiments will be carried out under contract at independent laboratories, such as those of the Agricultural and Food Research Council or Natural Environment Research Council that already have experience in deliberate release experiments. A major aim of the "Planned Release of Selected and Manipulated Organisms" initiative, he says, is to hasten the evolution of a framework for deliberate release, which is being constructed on a case-by-case basis by the Advisory Group on Genetic Manipulation. ☐

Genentech lose Protropin case

Washington

A LEGAL dispute between Genentech and Eli Lilly over recombinant human growth hormone has prompted a review by Congress of the Orphan Drug Act, the law intended to give commercial protection to companies making treatments for rare diseases.

Genentech filed a case against the Food and Drug Administration (FDA) in March, after the FDA approved Eli Lilly's form of recombinant human growth hormone, Humatrope, under the Orphan Drug Act (see *Nature* **326**, 231; 1987). Humatrope differs from Protropin by one amino acid, so it was considered a new drug by the FDA. But Humatrope is also used to treat dwarfism, and Genentech claimed that its marketing exclusivity for Protropin was being infringed.

Genentech last week learned that a complex legal strategy to protect Protropin from competition was a failure. Genentech sought to void both its own and Lilly's protection under the Orphan Drug Act, claiming that neither Protropin nor Humatrope was a "new drug", and that neither should be protected by the act. But a US Federal circuit court judge dismissed that argument, upholding the rights of Eli Lilly to market their drug.

Now the legislature is gearing up to clarify the Orphan Drug Act, to be introduced into the House on Tuesday. As *Nature* goes to press it is unclear whether the amendments will define the term "drug" in the context of the act, or establish a principle of "shared exclusivity", several drug manufacturers enjoying market protection for the same product for slightly different indications.

Carol Ezzell

French government shifts accent of spending towards industry

Paris

The 1988 budget proposals presented last week by French minister for research and higher education, Jacques Valade, confirm the government's intention to shift the balance between private- and public-sector science and technology research in France. While several measures are envisaged to encourage industry-sponsored research, growth in state-supported research establishments (*grands organismes*) has been clearly restricted.

University teaching departments, which lack the infrastructure to carry out research, do slightly better in the budget. Grants for university-based research increases next year by 14 per cent, although this is less than the increase for 1987 (25 per cent).

The overall science and technology research budget will increase by 8.5 or 10.5 per cent compared to last year (depending on whether pledged sums are included in calculations), but this includes both civil and defence spending. When civil research alone is considered, the increase is a more modest 3.4 per cent, to FF39,345 million ($6,503 million), against an annual inflation rate of about 2 per cent. Defence research spending, while less than in 1987, will still increase by 10 per cent to FF33,000 million.

Proposed grants to individual research establishments — still to be agreed by the Finance Ministry — vary. The Centre National de la Recherche Scientifique (CNRS), with its associated national institutes, and the Institut National de la Santé et de la Recherche Médicale (INSERM) — the two largest *grands organismes* — can expect increases of about 1 and 3 per cent, respectively. The Institut Français pour l'Exploitation de la Mer (IFREMER) will get an increase of about 2.6 per cent over 1987, while the nation's controversial technology 'shop window', the Cité des Sciences et de l'Industrie, at La Villette, Paris, will have its grant drastically cut by 11 per cent (see *Nature* **329**, 96; 1987).

The lack of government favours for the *grands organismes* is also seen in the negligible number of new posts for young researchers for 1988 — 150 overall, against 398 in 1987. Speaking to the press, Valade considered the creation of new jobs "an achievement", since none had been expected, but forgot to mention the loss of 350 posts at engineer, technician and administrator grades.

Unlike the situation in most Western countries, researchers taken on by the *grands organismes* have jobs for life. Because of a high intake of new researchers in the 1960s and early 1980s, when public sector research had a higher government priority, present research teams are relatively young and senior staff will retire at about the same time, in the late 1990s.

Unions representing researchers, as well as the independent advisory body, the Conseil Supérieur de la Recherche et de la Technologie (CSRT) argue that a much higher annual rate of job creation is necessary to offset the "massive" drop predicted for the end of the century. Valade, on the other hand, feels that "because growth in employment has been high it need not stay high" and wants to see posts liberated by mid-career researchers joining the private sector. Considerable financial incentives have been offered to researchers making such a move, and FF500 million of tax incentives are to be available to companies developing their research activity.

The government emphasis on commercially exploitable research is also apparent in a 50 per cent increase in grants to the Agence Nationale de Valorisation de la Recherche (ANVAR), set up to promote research and development in industry, particularly in association with public sector research groups. ANVAR's grant had previously been cut by 40 per cent when the present government took office last year, revealing a dramatic change in policy towards research.

Grants totalling FF930 million (compared to FF750 million for 1987) have been earmarked for the Fonds de la Recherche et de la Technologie (FRT), a research fund reserved for national priority research programmes. Valade announced the 11 rubrics for which tenders may be submitted (by private as well as public sector research groups): (1) biotechnologies, (2) nutrition, (3) medical research (including AIDS and retro-virus research), (4) human sciences, (5) technology and production, (6) electronics and information technology, (7) transport and civil engineering, (8) natural resources and the environment, (9) new materials, (10) new chemistry and (11) development.

Valade singled out research on high temperature superconductors as a prime example of an area where industry and the *grands organismes* should collaborate. Under the new materials heading of the FRT list of priorities, FF20 million ($3.28 million), will be available, in addition to existing grants to public sector physics and chemistry laboratories. **Peter Coles**

European data net under threat

London

Proposals by the West German telecommunications authority, the Bundespost, to impose usage-related charges on top of the costs of leased data lines would, if implemented, have "disastrous consequences" for European academic research, Dennis Jennings, president of the European Academic Research Network (EARN), warned last week. Such action by an individual national authority would undermine strategic research programmes and would be against the best interests of the European community, Jennings says. Last week EARN decided to give up four of the five international leased lines that it runs into West Germany. □

Franco-German agreement to tap hot rocks

Paris

French and West German geological establishments have signed an agreement to collaborate on an exploratory geothermic energy project at Soultz-sous-Forêts, in the Rhine valley near Mulhouse in Alsace. The first phase of the project, which should be completed in 1989, will be undertaken by the French Bureau de Recherches Géologiques et Minières (BRGM) and the German Bundesanstalt für Geowissenschaften und Rohstoffe, at a cost of FF50 million (£5 million). The European Economic Community is to contribute FF14 million to the costs from its general research fund, the rest coming from national research budgets — FF16 million from France and FF20 million from West Germany.

The geothermal gradient, around 3 °C per 100 m, usually makes it impracticable to exploit the Earth's heat energy at the surface, but at Soultz-sous-Forêts, geological anomalies have resulted in temperatures of 150 °C in the underlying granite at a depth of only 1,800–2,000 m. At this temperature the energy can, in principle, be used not only to heat water pumped into the drill hole, but also to generate electricity, using heat-exchanges at the surface. As the Rhine itself forms the frontier between France and Germany in this region, both countries would be able to benefit from the energy if it can be harnessed, hence the decision to collaborate.

Similar projects at Los Alamos in the United States and in Cornwall in Britain encountered serious difficulties in recovering hot water at the surface, but depths are greater than those in Alsace (3,500 and 2,500 m, respectively) and because this part of Alsace was also the site of exploratory drilling for oil, its geology is well documented. The disused wells and derricks will be exploited for the initial seismic, hydraulic and corrosion studies comprising phase I of the geothermic projects.

Peter Coles

Fifteen per cent solution to keep Britain in synchrotron club

London

BRITAIN's reputation as a fickle collaborator on international science projects has been further reinforced after last week's meeting of the European Synchrotron Radiation Facility (ESRF) council in Grenoble, France. Nine of the ten member states were able to give provisional commitment on funding the facility. Embarrassed UK delegates could not say how much money Britain will be able to afford, or indeed if it will remain a partner. Britain has until the end of the year to come up with a subscription fee acceptable to the other members.

Originally, five nations were involved in ESRF's £3 million foundation study, each member contributing a sum roughly in proportion to its gross national product: France (38 per cent), West Germany (28), Britain (15), Italy (15), Spain (4). Since the foundation phase started in April last year, five more countries have joined the venture, Switzerland and a 'Nordic consortium' of Norway, Denmark, Sweden and Finland. The Nordic countries will together contribute 4 per cent, as will Switzerland.

At last week's meeting, the ESRF council formally approved the foundation phase report, which covers an 11-year period, 6½ years for the machine's construction and 4½ for experimentation, at a cost of £350 million. The ESRF council wants work to start next year.

The council will meet in November and again in December, when final levels of funding will be agreed. Britain's contribution according to a formula based on gross national product should be of the order of 18 per cent. The other partners consider 15 per cent acceptable, but might not be prepared to tolerate a disproportionately small subscription fee from what is still seen as a wealthy nation. Britain's position depends on the outcome of the government's public spending review in the autumn.

The Science and Engineering Research Council (SERC) is keen to see Britain in ESRF. The present difficulties have arisen principally because SERC failed to entice France to enter a collaborative arrangement on the spallation neutron source, ISIS, at the Rutherford Appleton Laboratory. SERC had hoped that France would agree to take a 20 per cent stake in the facility. Discussions are still proceeding along similar lines with Italy.

Synchrotron users in Britain are lobbying hard for inclusion in ESRF at the level being requested. As with most international collaborative ventures, contracts for building the machine will be awarded to individual countries in proportion to their contribution, and the input and influence of the scientists using the facility will be similarly determined. Such advantages would be lost if Britain decided to join the facility once it had been built. Furthermore, ESRF's supporters say, the sums involved are, by comparison with other international projects, relatively small. At 15 per cent, Britain's contribution would be on average between £5 million and £6 million annually for the 11-year period, with the 1988 contribution possibly as low as £1.5 million.

As with Britain's involvement with the European Space Agency (ESA) and the European Organization for Nuclear Research (CERN), discussions on funding are plagued by uncertainty and patience on the Continent is wearing thin.

Meanwhile, a £1 million upgrading of SERC's own synchrotron source at Daresbury in Cheshire has been completed. The high brightness lattice is a modification of the 2 GeV synchrotron radiation source, increasing its brilliance by an order of magnitude. Inaugurating the new high brightness lattice on September 17, higher education minister Robert Jackson extolled the benefits of international collaboration on scientific projects. The irony of his remarks will not be lost on the beleaguered British officials at CERN, ESA and now ESRF. **Simon Hadlington**

Europe's half-hearted research budget

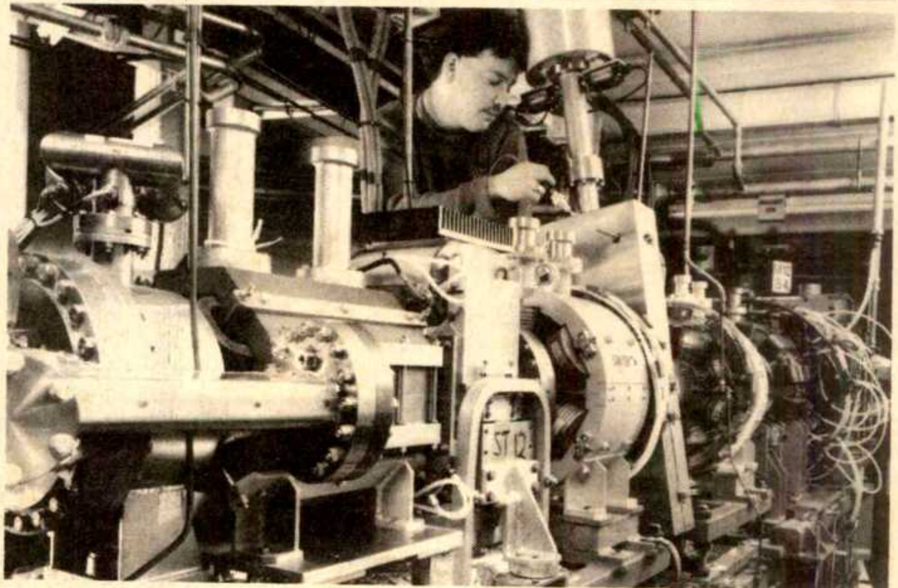
London

THE twelve Common Market governments were expected this week finally to approve the five-year Framework programme of research and development. The budget will be far leaner than the £7,300 million originally proposed, mainly at Britain's insistence that the programme in its original form was too costly and unfocused. The final figure is expected to be around £3,700 million, with the possibility of a further £300 million at the end of the year if community spending in other areas, notably agriculture, is brought under control to the satisfaction of all members.

Among the first casualties of the reduced research budget are more than half the projects selected as eligible for support under the latest phase of the BRITE (Basic Research in Industrial Technologies for Europe) programme. Last week 46 projects were selected for grant-aid worth £31 million. A total of 471 projects had been submitted for consideration, of which 250 were deemed eligible. A further 66 projects should receive £45 million after ratification of the Framework budget. More projects could have been supported had the original levels of funding been agreed, say officials.

The BRITE programme, launched in 1985 as a four-year project to increase the use of advanced technologies in traditional industry, now supports some 210 projects.

Simon Hadlington



*THE construction and, more controversially, the financing of Europe's synchrotron sources has long been an area rife with international horse-trading. At an early phase in the negotiations to build the ESRF at Grenoble, the British partners (SERC) were keen to gain collaborators to share in the costs of the Spallation Neutron Source at Daresbury, near Oxford. French budget cuts last year effectively spelt the end to such hopes (*Nature ***321***, 7; 1987), although now that the Daresbury source is in operation, the facility is open to experimenters from other countries who pay for time on the machine and related services. One of the refinements to the Daresbury synchrotron now upgraded is a smaller more concentrated beam, achieved by adding new focusing magnets (centre of photo above) to every straight section of the storage ring.*

Scientists take share of blame for this year's poor Soviet harvest

London

THE lateness of the harvest this year is already causing heart-searching and rethinking among Soviet agricultural planners. One of the last major events of the Brezhnev era was the Food Programme, issued in June 1982, which was meant to ensure the long-term ability of the Soviet Union to feed its population by the year 2000. Although some lip-service is still paid to this programme, there now seems to be a tacit assumption that it will not materialize in its original form — and, indeed, one of its major features, the southward diversion of the north-flowing Siberian rivers, has been shelved.

Nevertheless, the main premise of the Food Programme persists — scientists should be more efficiently mobilized in the service of agriculture. A resolution of the All-Union Council of Ministers and Central Committee of the Communist Party of the Soviet Union in August took the same line. As part of the general "restructuring" of science on to a cost-efficiency basis, it offered financial incentives for research groups and institutes which contribute to increased productivity in agriculture. The profits from such innovations are, according to the new resolution, to be shared between the researchers and the farms which put the new methods into operation — thereby countering one of the main objections of scientists in past years, that workers on collective and state farms, who for some years now have enjoyed guaranteed wages, were not greatly interested in the changes and extra efforts involved in trying out new crops or procedures.

The August 1987 resolution, "on improving the scientific back-up for the country's agro-industrial complex", has a more sophisticated approach to science than previous decrees. Instead of adopting the Brezhnev approach to the "chemicization of agriculture", which advocated the use of pesticides and mineral fertilizers, it speaks of an "integrated" approach to pest control, using "ecologically safe" plant protection agents, including natural predators. Fertilizers are to be applied in accordance with growth and development, and there is to be more theoretical research into the mineral nutrition of plants. Instead of grandiose irrigation schemes the emphasis is on efficient use of existing resources, including drip-feed and synchronous pulse irrigation. Now more productive strains of livestock and plants are to be developed, including adaptations for the arid zones.

All this implies, that the scientists have been failing in their task — and *Pravda* said as much in a leading article on the new resolution. The resolution "defined" the All-Union Lenin Academy of Agricultural Sciences as the "supreme" centre for the "comprehensive scientific back-up programme" but, *Pravda* said, it has failed to assume a leading role in the necessary experimental and implementation work. The All-Union Research Institute of the Electrification of the Agricultural Economy and the All-Union Research Institute of the Physiology, Biochemistry and Nutrition of Agricultural Livestock were singled out as examples of "low resultativity". In particular, said *Pravda*, the new programme would necessitate increased efforts from the higher education sector, including major changes in training and updating courses and the establishment of special faculties for grading the qualifications of agricultural scientists and lecturers.

Two weeks ago a feature article in *Pravda* by Lenin-Prize winner G. Georgiev, which was outspoken even for the current era of *glasnost*, pinpoints the major legacy of Lysenkoism as an intense shortage of trained research personnel, a lack of proper appraisal of research results, and the absence of a small network of top-level laboratories (Georgiev spoke of 20–30) which would act as pacesetters for the rest. (What, one is left wondering, became of the massive emergency investment in molecular biology and genetics authorized in May 1974, which was intended to supply just such a base?)

Other scientists maintain that they have been working for agriculture on the lines now proposed for years, but that their results have been ignored or misapplied. In a recent Soviet television documentary, the president of the Moldavian Academy of Sciences, Dr Aleksandr Zhuchenko, spoke of major top-level errors in land-use, caused by politicians overriding the scientists' recommendations. In one case, he said, the All-Union Ministry of Land Reclamation and Water Resources had authorized plans for an irrigation system in the face of protests from scientists who pointed out that the water in question was unsuitable and would damage Moldavia's black soils. Zhuchenko further questioned the official Soviet approach to farm production targets. Instead of aiming at high yields in favourable years — and then writing off subsequent low results as due to adverse weather — the agricultural planners should aim at a balanced programme, producing good yields every year, including dry years, and should eschew doubtful management practices that can produce a one-off record crop, but from which the soil will then take ten years to recover.

Vera Rich

Fish psychology is big business

Tokyo

JAPANESE fisheries scientists are resorting to psychology to keep fish under their control. An experimental station in the southern island of Kyushu is applying electric-shock treatment to keep fish within a netless farm, while another is using the Pavlov technique and sound to confine red sea bream in an open bay.

Fish farming is big business in Japan with an annual production of just over one million tons — enough to satisfy the fish consumption of France. The electric fish farm is only in the very early stages of development. Hitachi Zosen Corporation, a shipbuilding company, and Kumamoto Prefectural fisheries station are testing the system in a tank. The farm is defined by three rings of metal stakes; the central ring is electrified. The distance between the central and outer ring is short, creating a strong electric field. Fish confined within the inner ring encounter an electric field that increases in strength outwards if they stray beyond that ring. Finally they are stunned before they can escape. When the fish regain consciousness on the sea floor they usually swim back to the inner circle. The electric farm avoids the problem of clogged nets which causes reduced oxygen supply and disease.

The sonic fish farm at Oita Prefectural fisheries station in Kyushu is at a much more advanced stage and employs quite the opposite psychological approach. Red sea bream are raised from eggs in tanks and then netted enclosures in the sea to the 3-cm stage. They are then conditioned to associate sound with food. Sounds are played to them at the time of feeding. At first a piano piece composed by an Oita University professor was used, but the fish did not like the variation in frequency. Bongo drums were not to their liking either. In the end, a plain 'pooh pooh' at 300 hertz and 50 decibels did the trick.

After 80 days of sound conditioning, the fish, now 10 cm long, are released into the bay where all the latest high-tech equipment is brought to bear to prevent the sea bream from swimming away.

One of the great advantages of the ranch is that the costs of feed and manpower are greatly reduced — 70 per cent of production costs of conventional fish farms is taken up by food and labour. And even although the 20 per cent recovery for released fish is less than that of a conventional farm (80–90 per cent), Yasumura estimates that production costs per kg for a sonic fish farm will be less. And as the fish look and taste like their natural wild cousins, he expects them to command twice the price of the conventional farm variety.

David Swinbanks

Acid rain report assailed as misleading

Washington

A NEW US government report on the causes and effects of acid rain is, depending on who you speak to, either a careful assessment of currrent scientific uncertainty or a misrepresentation of a serious problem. The report is the product of almost seven years of deliberation by scientists working on behalf of the National Acid Rain Precipitation Assessment Program (NAPAP), representing a number of government agencies. Its four volumes are acknowledged even by detractors to contain a comprehensive and mostly sound study of the sources of pollution, atmospheric chemistry, damage to lakes and forests, and potential methods of controlling emissions. But critics, such as Michael Oppenheimer of the Environmental Defense Fund, call the executive summary "skewed" in its summing up of the evidence.

Two particular disagreements stand out. Surveying all of the United States, the report declares that the number of lakes acidified to a damaging degree is no more than a few per cent of the total. NAPAP deems a lake unacceptably acidified only if its pH is 5.0 or less, when fish die and direct economic loss follows, but according to Jim Gibson of Colorado State University, there is obvious disruption to the food web, with concomitant loss of fish, at a pH of 6.0. The NAPAP report also emphasizes that no harm to seedlings has been demonstrated from acidic precipitation at "regional ambient levels", a statement which, says Oppenheimer, is true but misleading; extensive cloud cover of pH as low as 3.2 has been found in New England and it is these episodes, rather than average immersion at higher pH, that are responsible for forest damage in the north-eastern United States.

Many of those unhappy with the report see too much in it of the beliefs of NAPAP's chairman, J. Laurence Kulp, a geochemist. But Kulp defends the report, saying that the executive summary represents a consensus of the authors of each chapter and of senior scientists from all the participating agencies. He says that lakes naturally exist with a wide range of acidity, and that a pH of 5.0 is when biological activity falls off most sharply. David Streets, of Argonne National Laboratory, was "entirely comfortable" with the parts of the report to which he contributed. Bob Downing, a spokesman for Kulp's office, did admit that NAPAP had "learned the hard way" the perils of summarizing a hefty report in one-page chapter synopses.

Described by his opponents as "strong-willed", Kulp was apparently given his position at NAPAP for strength of character rather than political leanings. But his panel's report carries undoubted political weight in the dispute between Canada and the United States over the effects of acid rain. Ross Glasgow, First Secretary for the Environment at the Canadian Embassy in Washington, described the NAPAP report as a "very unhelpful" contribution to the acid rain debate, and contrasted it with laudable US action on atmospheric ozone, where the scientific evidence is less clear. But so far there has been no government comment on the NAPAP report, and Glasgow believes that strong criticism of it within the United States may already have undermined its credibility.

Canadian scientists were given a preview of the report before publication, and made known their worries that by neglecting the higher acidities in Canadian surface waters the magnitude of the problem was not fully expressed. And although, says Glasgow, the NAPAP report was specifically a US study, its omission of evidence from Canada and Europe lent bias to its conclusions.

Kulp himself is about to resign his chairmanship of NAPAP having completed what he regards as a "major milestone". NAPAP, established in 1980 with a ten-year mandate, will continue to compile evidence. A new chairman has not been announced, but both Kulp and Downing expect the programme to carry on in the same vein.

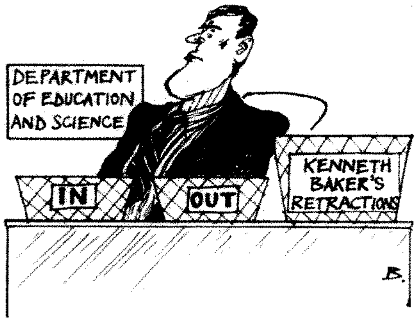
David Lindley

Britain's universities outshone by US fund-raisers?

London

EMBARRASSED officials at the Department of Education and Science (DES) are trying to play down an off-the-cuff remark by education secretary Kenneth Baker which implied that British universities were not putting in enough effort to raise funds from private sources. Speaking in Chicago during a tour of the United States last week, Baker was reported on BBC radio as saying that more British universities should follow the example set by their US counterparts by employing full-time fund-raising staff. According to the report, Baker said that Southampton was the only British university to do this.

His remarks prompted an immediate and angry response from the universities. The Committee of Vice-chancellors and Principals was quick to point out that nearly half of Britain's universities employ fund-raisers, and that universities have doubled their income from outside sources in four years. DES officials — also faced with objections from British school teachers upset at Mr Baker's apparent preference for US teaching methods — claim that Baker was inaccurately quoted and that he mentioned not only Southampton but also Oxford as two examples of British universities that have full-time fund-raisers. According to an official, Baker was merely airing his view that fund-raising was "a good thing that ought to be encouraged". The universities are aggrieved because they feel they have responded positively to the harsh economic climate by actively seeking non-government support. In 1985–86, Salford University earned nearly a quarter of its total income of £28.9 million from the open market. A spokesman from Kent University, which is currently seeking a Development Officer to launch a major fund-raising campaign in 1990, says that Baker's remarks will "reinforce public prejudice that the universities are lying on their backs waiting to be spoon-fed with government money".


DEPARTMENT OF EDUCATION AND SCIENCE
IN
OUT
KENNETH BAKER'S RETRACTIONS
B.

Warwick University, which earned 15 per cent of its £37.3 million income from non-government sources in 1985-86, points out that it built the largest arts centre outside London with mainly private funds. London University's Imperial College has recently appointed a Director of Public Affairs, with responsibility for fund-raising. In general, most British universities do not have a strong tradition of raising funds from alumni, although it is a potential source of funds receiving considerable attention. University fund-raisers say that despite Baker's exhortations, funds from private sources will not materialize overnight. Furthermore, it is argued, the government itself could help by introducing more favourable tax incentives for corporate and charitable donations. Perhaps the biggest disappointment over Baker's alleged comments is that the Secretary of State is apparently unaware of the universities' efforts to do as the government has told them.

Simon Hadlington

Chaos and complexity reign in the New Mexico mountains

Santa Fe, New Mexico

BARELY three years old, the Santa Fe Institute (SFI) can hardly be accused of having modest goals. "We are trying to define the scientific and educational agenda for the twenty-first century", says David Pines, University of Illinois physicist and co-chair of the SFI's science panel. The immediate challenge for the institute is to find funds among traditional sources for some non-traditional ideas.

From the outside, SFI is unassuming. It is located in a converted convent, a brief walk from the state capital, down a road famous for its art galleries and craft stores. But inside there have been interesting goings on. Throughout the past summer, SFI has hosted a series of meetings and workshops to begin the process central to its goal of helping to catalyse new, interdisciplinary approaches to understanding complex systems.

Gell-Mann — who says SFI should provide a nurturing environment for those looking to cross traditional boundaries.

A recent example was a meeting dubbed "Evolutionary paths of the global economy", co-chaired by physicist Philip Anderson of Princeton University and economist Kenneth Arrow of Stanford University — both Nobel prize winners. Sponsored by Citicorp and the Russel Sage Foundation, the meeting brought together economists and physical and biological scientists who have used nonlinear dynamic models to study adaptive paths.

Other intriguing topics discussed this summer include the matrix of biological knowledge — an attempt to bring the tools of computer science and artificial intelligence to bear on the increasing volume of biological and clinical data — theoretical immunology, and a meeting this week on computational approaches to evolutionary biology.

SFI president George Cowan, who is also a senior fellow at nearby Los Alamos National Laboratory, says there are many visions of how the institute might evolve. Physicist Murray Gell-Mann of the California Institute of Technology is one of SFI's founders, serving now as co-chairman of the science board and a member of the board of trustees. He sees SFI as providing an alternative to universities where it is difficult to foster interdisciplinary interactions. Gell-Mann says interdisciplinary centres are often relegated to the basement of a university-owned but unused Victorian mansion, with staffs forced to survive on short-term grants, shunned by the academic departments to which the centres nominally belong.

SFI currently maintains a small permanent staff. If money is found to support expansion, the core will stay small, but there will be a coterie of visiting faculty and graduate and postgraduate students. SFI has been surviving on foundation grants and private contributions. But word is expected shortly on two multi-million dollar grant applications, one to the National Science Foundation and one to the Department of Energy. There is apprehension among SFI officials that the grants may be difficult to get, since the institute does not fall into a convenient category.

The proximity of the Energy Department's Los Alamos National Laboratory has a significant influence on SFI. Many of SFI's board members are affiliated with the national laboratory, and SFI co-hosted with the Los Alamos Center for Nonlinear Studies a workshop on artificial life.

There is no unanimity among board members about the specific paths SFI should take. But those actively involved with the institute radiate an excitement about its potential, and they believe that through the interaction of talented people there may well arise new visions of how the world is put together. **Joseph Palca**

Eastern bloc exchanges may benefit from new rules

London

THE inflexible regulations and complicated red tape that have made life difficult for Soviet scientists working abroad should be eased, the authorities claim, with the recent changes in Soviet rules for travel. Existing Soviet procedures on foreign assignments for scientists and technologists have come under strong criticism of late — both from a senior Soviet diplomat and the scientists themselves. Interviewed by *Izvestiya*, Strizhkov, the Deputy Economic Counsellor at the Soviet Embassy in Sofia, said that although 376 Soviet specialists are now established at Kozlodui, where they are officially assisting the Bulgarians in the construction of a nuclear power station, many of them are not required at the present time.

The Soviet practice is to send such persons abroad on a two-to-three years' posting; however, many of them are actually required on the site only for a series of relatively short periods, and, if the paper-work permitted, could easily return to the Soviet Union in between. Unfortunately, no one will send them home, since there is no guarantee that they will be able to return when required. And, if a particular specialist has completed his term at the site, he has to go back to the Soviet Union, even if the task for which he has come has not been completed.

Plans for the supply of Soviet specialists to such projects are laid down in advance and until now there has been no provision made for change of schedule or emergency. *Izvestiya* quoted a case where problems arose with equipment supplied to Kozlodui by a Hungarian plant, a West German firm and the Soviet Tulaelektroprivod trust. Hungarian and West German trouble-shooters were on site within a week; the Soviet team arrived six months later. The latest changes, it is hoped, will make such "emergency visits" easier.

As well as keeping specialists idling abroad, long-term postings mean the need to support a considerable infrastructure: the Soviet colony at Kozlodui usually contains 800 to 1,000 persons, with inevitable problems of housing, transport, medical services, children's schooling and so on.

There is considerable pressure from the Kozlodui Soviet colony, *Izvestiya* said, for such "fraternal assistance" to be organized on a task-basis rather than a time-basis. Such a view-point is likely to receive considerable support from Soviet industrial managers. Until now, managers have been reluctant to have their specialists and scientific staff coopted, since although the host country paid for all technical services provided by the Soviet Union, none of this money filtered back to the enterprise which provided the specialists. Under the new Law on the State Enterprise, adopted on 1 July, all foreign "economic activity" including the "export" of consultancy and technical services, will be considered as part of its overall performance. Enterprise managers will therefore wish all such foreign "activity" to be planned on a basis of cost-effectiveness, and, *Izvestiya* argues, will favour any scheme which gets the specialist back to his or her regular job as soon as possible. **Vera Rich**

Aleksandrov is still missed by his collaborators in ENUWAR, and at their Bangkok meeting in February they renewed the consensus on the predictions of climate change made in the SCOPE report which acknowledged the insight given by the limited one-dimensional model of Aleksandrov and Stenchikov.

FREDERICK WARNER
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VERA RICH REPLIES — The suggestion that Aleksandrov may have been restricted during his final visit to the Livermore Laboratory was originally made from the Soviet side, and confirmed in telephone conversations by his US hosts, who clearly felt awkward about the situation. I made no suggestions about the propriety or otherwise of such surveillance — which, according to Aleksandrov's hosts, was considerably less than that which I would accept as normal in working in a socialist-bloc country. □

Hungary as neurosciences audit

SIR—The meeting of the International Brain Research Organization (IBRO) — the second World Congress of Neuroscience, held in Budapest from 16 to 21 August — with some 3,000 participants, makes an interesting testing ground for international comparisons on relative scientific standing. I have therefore analysed the oral and poster presentations listed in the conference programme by geography of origin. In the recent past British contributions to neuroscience have been pre-eminent, and one might have expected a high British profile at the meeting. Because the meeting was held in Hungary, it could be presumed that access was relatively easier to Europeans, East and West, and somewhat less to participants from other continents, than would have been the case with another venue

The oral presentations were all invited, and therefore indicate the 'visibility' both of subject areas and the leading researchers within them. Of 612 listed papers, only 59 — just under 10 per cent — were from UK laboratories. Thirty-seven per cent were from East or West Europe, 37 per cent from the United States, a miserable 3 per cent from the Soviet Union and 13 per cent from the rest of the world, predominantly Japan, Canada, Australia and Israel.

Poster presentations were self-contributed, and as far as I know all those submitted were accepted. In one full day's set of 490 posters, just under 20 per cent of the entire meeting, only 23, or fewer than 5 per cent, were from UK laboratories. Thirty-one per cent were from East and 34 per cent from West Europe, 13 per cent were from the United States, fewer than 5 per cent from the Soviet Union, and the rest of the world contributed 12 per cent.

The first conclusion to be drawn is that the high visibility of contributions from US laboratories, leading to their domination of the oral sessions, can only enhance the well-known Matthew effect by which to him that hath more shall be given.

Second, the continuing prestige of senior UK scientists ensured them a significant presence among the oral presentations, but the weakness of the underlying position of UK neuroscience is shown by the small number of poster contributions. The more junior British scientists either had little to say or, more likely, were simply unable to raise the funds to attend. These two observations support the view that if European science is to increase its own visibility, it must reinforce its own cooperative mechanisms rather than stand so passively in awe of US achievements.

Third, the tiny representation of Soviet scientists even at an Eastern European meeting of a Unesco-sponsored organization to which the Soviet Union is affiliated, and despite the distinguished Soviet neuroscientific tradition, may say something about how far *glasnost* still has to go.

Fourth, it is a mockery of the concept of internationalism when a 'world' meeting boasts no contributions from Africa and only a handful from India, China and Latin America. IBRO's world remains overwhelmingly white.

Fifth, the gendered pronoun I used to define the Matthew effect was no slip of the pen. I estimate that 20–30 per cent of those attending the meeting were women. A significant number of the posters had women authors and women presenters. But women speakers in the oral sessions were conspicuous by their absence. Given the chance to choose speakers for these sessions, it seems the predominantly masculine group of organizers and chairs could see only men.

The pattern of this meeting is likely to be mirrored in the thousands of other international meetings held each year. And it is precisely because of this — and also because in this case the organizing body, through its ultimate links with the United Nations, has legitimate claims to universalism — that the experience is worth pondering, rather than being regarded simply as part of the natural order of the scientific world. At the least, positive efforts, nationally and internationally, are needed to help redress the imbalances that this simple numerical exercise reveals.

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Aleksandrov's visit

SIR—Vera Rich (*Nature* **327**, 544; 1987) writes that SCOPE may have put restrictions on Vladimir Aleksandrov during his US visit in January 1985. Not so. SCOPE-ENUWAR has given every opportunity for speedy communication of new information, incurring criticism for not going to recognized journals with standard refereeing. I know Aleksandrov was not allowed direct access to Cray computers during his US visit.

Aleksandrov visited Japan in February 1985 for the SCOPE-ENUWAR workshop and said he was able freely to discuss climate modelling during his US visit. In Japan he openly met scientists from Livermore, Colorado State University, NCAR Boulder, NASA-Ames, France, West Germany, Australia, Sweden and Japan. He talked to me about personal problems of his wife's health and up to the time of his disappearance was sending medical reports for study in England with a view to arranging treatment.

Argentine trials

SIR—K.S. Jayaraman (*Nature* **328**, 287; 1987), refers to a statement by Dr S. Ramachandran that "Argentine controversy (over rabies vaccine) arose because Wistar failed to inform the government".

The published facts (*Nature* **326**, 636; 1987) reveal that: (1)The trial in Argentina was carried out by the Pan American Health Organization not by the Wistar Institute. And (2) the "controversy" arose because an Argentinian scientist decided to play politics instead of considering the trial as an important step in evaluating a vaccine which may represent the best way of preventing rabies infection.

HILARY KOPROWSKI

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Human embryo

SIR—Your suggestion (*Nature* **327**, 87; 1987) to ban the unnecessary word "pre-embryo" (an embryo before implantation) is well taken. At the Carnegie Collection, in human embryology, the term embryo is used for the "human offspring in first eight weeks" (*Concise Oxford Dictionary*). Hence prenatal life is subdivided into merely two periods: embryonic and fetal. Terms we have discarded for the human embryo include ovum (used incorrectly for everything from unfertilized oocyte to three-week embryo), 'egg' (best reserved for a nutritive object sometimes seen on the breakfast table), and blastula, blastocoel, blastopore, gastrula, branchial, all of which are inappropriate.

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Physics successes

Sir—John Maddox made an unfortunate remark in his article (*Nature* **328**, 375; 1987) on the meeting at Bristol to commemorate the 1947 discoveries of the pion and the V-particle. I did not detect in the nostalgia of the Bristol meeting any indication "that these developments should have turned out to be the last important contributions of British experimentalists to the field . . .". On the contrary, I believe they were simply the first.

British physicists have taken a prominent part in all the major experiments at the European Organisation for Nuclear Research (CERN) and Deutsches Elektronen-Synchrotron (DESY) over the past decades (University College London and Oxford in the discovery of neutral currents in 1973; Birmingham, the Rutherford Appleton Laboratory and Queen Mary College in the discovery of the W and Z particles in 1983; Manchester, Lancaster, Oxford and Imperial College London in the discovery of gluons in 1979). They also played a leading role in neutrino and muon scattering experiments at CERN over the past decade, identifying partons with quarks and providing the first quantitative support for the theory of interquark forces (quantum chromodynamics). Even the Kendrew committee paid tribute to the quality of their work.

Because the people concerned work in large international teams, the role of individuals is not as clear as it was in 1947. But to dismiss the post-1947 achievements of the UK particle physics community is indefensible and just plain wrong.

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Geology departments

Sir—The University Grants Committee's Report of the Earth Sciences Review concludes that resources in this area in the United Kingdom are too thinly spread and that significant benefits would result from their concentration into a smaller number of large departments. Although several reasons are given why research productivity might be favoured by large size no firm evidence is presented. To shed light on this issue, I analysed the published research output and citation rates of 33 university departments of geology or earth sciences in UK universities.

An indicator of the quantity of published work of each department was derived from the *Science Citation Index* (SCI). Citations are collected from a full coverage of every item published in 3,250 journals as well as selective coverage of other publications. The Corporate Index volumes of SCI provide a convenient source of information classified by university and department. A count of substantial items was made for the three years 1982 to 1984.

The information on the volume of publications was supplemented by an analysis of citations, allowing distinctions to be drawn between work that has exerted a significant impact on a research field and that which has not. As citations are listed by individual, a list was made of names of individual members of each department as given by the *Commonwealth Universities Yearbook* for 1985; only full-time academic staff were included. For these individuals, citations in SCI were counted for the period used in the analysis of publications. Information on number of faculty in each department was also used to calculate *per capita* rates for publications and citations and as the indicator of size.

The correlation coefficient for the relationship between size of department and numbers of publications per member of faculty was 0.112 and that with number of citations per member of faculty was 0.256. Neither is significant at the 0.05 level. The results therefore provide no evidence of any significant relationship between research productivity (as measured here) and departmental size. Whatever the other merits of a concentration of resources, the view that this would enhance research productivity should be treated with some scepticism.

Graham Bentham

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South African view

Sir—As a South African expatriate I am struck by the muddled thinking in the recent correspondence over an academic boycott. Analyses such as those of J.G. Wilson (*Nature* **328**, 288; 1987) and Max Wallis (*Nature* **328**, 374; 1987) lead nowhere except back to their original ideological premises.

Like many South African-born families lucky enough to have the choice, my family left (in 1975) when it seemed that, whatever political options were chosen, there was no acceptable solution. While opposed to apartheid in principle, and abhorring many of its practical evils (the separation of black families, imprisonment without trial, the ultrahypocritical actions of many whites), we saw no political alternative to which to aspire.

The simplistic approach of 'one man, one vote' was not tenable. Like it or not, lumping together large groups of people with diverse cultures creates a sure-fire setting for political nightmares, as is evident in many of the world's worst trouble-spots. Although W.D. Stein (*Nature* **328**, 374; 1987) claims this would not be as catastrophic for blacks as for whites, the history of independence in Africa suggests otherwise. This is not to argue that majority rule should be implacably resisted — it is only wise, when faced with the inevitable, to prepare for it. One must, however, be an especially confident idealogue to demand particular changes regardless of the trauma they invite.

These feelings support two conclusions: (1) there are no easy options for the white electorate in South Africa and (2) boycotts will not force whites to choose political platforms that serve no one except the next tribally based clique to assume the reins of government.

The question for outsiders thus boils down to a personal view of how to ease the tribulations that South Africans of all colours must undergo in the transition to majority rule. At present, this means adopting tactics that temper, as far as possible, the more brutal and reactionary aspects of the Nationalist regime. It argues for a strong dose of pragmatism.

Boycotts have had a significant political impact on South Africa. In sport, they may have done some good by forcing white electors to recognize the repugnance with which apartheid is viewed by nations they would call friends. In trade and economic cooperation, the issue is more difficult because black workers are more immediately affected than whites.

But what can be achieved by an academic boycott? The state the universities in South Africa, as in most places, is of virtually no concern to people in the street. They could hardly care less about the scientific well-being of academics. Neither, therefore, do the politicians care a rap. Nor will a boycott by scientists prevent South Africans from obtaining the technology they require. Yet the damage to liberal thinking in South Africa by isolation of the universities would be profound.

Peter M. May

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Sir—While it is probably inadvisable for a resident to comment on the views expressed in the recent correspondence following your article on the academic boycott of South Africa, I should like to record that in my second-year organic chemistry class this year there are 24 Indians and 5 Africans, the balance being 52 of European origin.

A few years ago, the class was of a similar size, but completely European. Although we are not allowed to increase the size of our intake, the racial composition is changing rapidly.

D.A.H. Taylor

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The Antarctic cornucopia

New results from a Soviet–French collaboration highlight the benefits to be gained from polar research, as well as a US scientific deficit.

THOSE who have spent time in Antarctica do seem to be something of a breed apart, not least because they generally return to 'civilization' itching to depart southward again at the earliest possible opportunity. This phenomenon must stem from some personal experience denied to less hardy mortals — the memory of a brilliant landscape of white and blue beneath an incomparably clear atmosphere, perhaps, or the attainment of as perfect a silence as nature will allow. A more mischievous speculation would dwell on the breathing space for misogynists provided by those national surveys that have up to now been staffed exclusively by males (though even the British Antarctic Survey may remove that particular iniquity soon).

Such superficial attractions are easily matched by other considerations. The political advantages of Antarctic research were amply highlighted by the sudden conversion to the cause by the British Prime Minister following the Falklands war. Other attributes of the region are self-evidently attractive to scientists: the Antarctic circumpolar currents which serve as both source and sink to deep ocean flows, of potentially crucial significance for climatic change; the proximity to an uppermost atmosphere linked directly to interplanetary phenomena by a downward-dipping geomagnetic field; a unique ecosystem; and, of course, a stratosphere whose dynamics and chemistry have now shone a red light at the chlorofluorocarbon industry.

But as a source of fascination the ice sheets are hard to beat. Their ponderous dynamics are unusual enough — the coupling between gravity, thermal flow and temperature-dependent rheology has so far rendered it impossible to solve several fundamental problems. For example, might a combination of rapid accumulation and bottom melting lead to a catastrophic surge? Is there a natural resonance within the combination of ice sheets, oceans and atmosphere, that can account for the strong glacial response to the tiny variation of incoming solar radiation caused by the 100,000 year Milankovich orbital ellipticity cycle? Progress is hindered by the effort it requires simply to measure in the field such fundamental quantities as ice flow velocities.

Such experimental and theoretical problems posed by the ice sheets have not, of course, prevented their fruitful exploitation, as three Franco–Soviet papers on the Vostok ice core in this issue amply demonstrate (pages 403–418). The flow properties are sufficiently well known, and the past annual snow accumulations sufficiently well inferred, for it to be possible to date ice (albeit with significant uncertainties) as one probes downwards. And as snow is compacted beneath successive falls, air within it eventually becomes trapped in bubbles. So if due allowance is made for the several decades such entrapment takes, one in principle has a direct chronological record of atmospheric composition and — thanks to stable isotopes and their various fractionations — of climate stretching back as far as the sheets permit.

The Vostok core is the deepest to have been drilled. Its 2,200 metres extend back over 160,000 years through the ice age that ended about 8,000 years ago and into the previous one. Of course, deep-sea sediment cores can extend over a million years, but they can be far harder to interpret given the combined influences of climate and oceans on the indicators that they contain. As it is, the new Vostok record of carbon dioxide and temperature provides crucial information for those wishing to assess the relative importance of greenhouse effects on past climates, as Eric Sundquist explains on page 389.

The engineering skills required for deep ice drilling are too easily forgotten. Conventionally, drills have used rotating blades around the cylindrical sampler base to cut into the ice. At Vostok the Soviets have perfected a thermal technique whereby the base of the 8 m × 10 cm tube is electrically heated, penetrating the ice as it melts without damaging the sample.

As one might imagine the technique is not without its difficulties, quite apart from those arising from the Antarctic weather. Simply keeping the hole vertical requires carefully controlled descent, while the meltwater produced by heating has to be rapidly recovered to prevent refreezing and distortion. Should the drill become jammed for any length of time the inexorable flow of ice will deform the hole, making retrieval impossible. And complications arise from the need to balance glaciostatic pressure by filling the hole with a fluid, such as alcohol, which can withstand the −55°C average surface temperature.

Small wonder, then, that several holes were attempted (as can be seen on the cover of this issue) and that the core took from 1980 to 1985 to be extracted. The current hole has now had to be abandoned but a new hole is to be started soon, potentially reaching a further kilometre or more into the ice. At such depths an annual layer may be only 1cm thick.

A look at the background of such drilling might repay a historian of Pleistocene and Holocene science. The range and the grandiose implications of the data that can be retrieved from ice and sea cores tend to eclipse the inevitably more gradual progress made by those concerned with continental records. This seems to have been mirrored by the comparative support for the respective communities, at least in France — a situation that seems unlikely to change given the selective policies adopted by the committees responsible for allocating money.

In the international context one can only express surprise at the low profile recently achieved in this area by the United States. Most glaciologists would identify Willi Dansgaard of Denmark, Chester Langway of the United States, and Hans Oeschger of Switzerland as the prime innovators of glacial palaeostudies, with US expertise in drilling being particularly crucial in the 1960s for the path-breaking Camp Century core in Greenland, for example. But although the United States has continued to support drilling activities of its own and of other countries, scientific returns within the US community have not maintained the early prominence, and Western Europeans, particularly from France, Denmark and Switzerland, now dominate the literature.

No doubt the National Science Foundation and the community share responsibility for this odd state of affairs, and the foundation is quick to point out that a major programme of Antarctic and Greenland drilling is about to commence. The danger is that such a programme may lack a sense of focus, given the diffuse character of current US involvement. Two factors may be particularly relevant. In each of the successful European countries, efforts are concentrated within multidisciplinary glaciological laboratories. And two particularly successful international enterprises — that involving the three individuals above on the one hand, and the French–Soviet work at Vostok on the other — originated in, and have succeeded as a result of, close but purely informal collaboration.

Philip Campbell

Archaeology

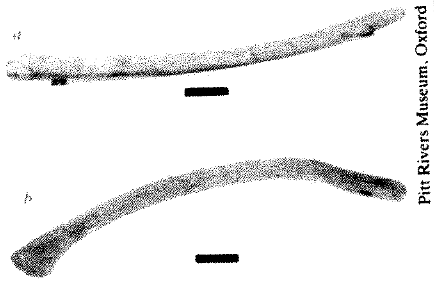
Return of the Euro-boomerang

Paul G. Bahn

PAWEL Valde-Nowak and colleagues on page 436 of this issue[1] report the discovery of a curved piece of mammoth tusk in a cave in southern Poland. They claim that the tusk is about 23,000 years old, and that it is the oldest boomerang in the world.

In the minds of children and adults alike, the boomerang is considered as uniquely Australian as the kangaroo or the vegemite sandwich. It has certainly been part of the Australian scene for a long time, as shown by the three complete wooden specimens from Wyrie Swamp, South Australia, which are 9,000 or 10,000 years old[2].

But boomerangs and 'kylies' (killing-sticks) have been found on five continents: some cave paintings in North Africa, for example, dating to perhaps 7,000 BC, are thought to depict an object of this kind, and boomerangs with gold caps at either end were found in Tutankhamun's tomb, dating to the mid-second millennium BC. Some form of throwing stick is also known to have been used by groups as far-flung as Eskimos, Hopi, Polynesians and Indians. In most regions, the invention of the bow and arrow eventually made the technique obsolete[3].



Pitt Rivers Museum, Oxford

Ancient Egyptian throwing sticks or boomerangs (from a, *XII Dynasty Thebes;* b, *XII Dynasty or later). Scale bars, 5 cm.*

In Europe, there have been finds both from the Iron Age (an oak specimen of 300 BC, preserved in a bog in Holland[4]) and from the Mesolithic (a find in Jutland from a settlement of about 5,000 BC). A piece of mammoth bone from an Austrian site was claimed to be a fragment of an Upper Palaeolithic boomerang, but its date and its identification are far from certain.

Valde-Nowak and colleagues think their new find, from a cave in the Oblazowa Rock in southern Poland, is a complete boomerang. It was well stratified with animal remains and with stone and bone tools attributed to the Pavlovian culture.

Not all curved objects, however, are necessarily boomerangs. The old joke — what do you call a boomerang that doesn't come back? A stick — is of relevance to this discussion. Killing sticks are far more numerous than boomerangs in both prehistoric and historic times, and probably predate the boomerang. The chances are high that the remarkable aerodynamic properties of sticks which happened to be curved were noticed and exploited independently on many occasions.

The only way to be sure that a curved object is a boomerang is to try it out, but few scholars would contemplate hurling prehistoric specimens into space to see if they come back! The best method is therefore to experiment with a cast or a replica, as has been done successfully with a plywood copy of the Dutch specimen, and with a replica of one from the eleventh Dynasty of Egypt[4]. But there is always the problem that many such prehistoric objects have been warped or damaged during the time they were buried.

No such experiment has yet been done for the Polish find, and meanwhile Valde-Nowak *et al.* are basing their claim on its shape, its curvature and the flattening at both ends. It has a span of 70 cm, is up to 6 cm wide, and up to 1.5 cm thick (see their Fig. 1 on page 437). One side preserves the external, convex tusk-surface, whereas the other has been polished almost flat. On the evidence of morphology, therefore, the object certainly makes a plausible boomerang, and the find is of potentially great importance for our knowledge of palaeolithic techniques of exploiting game, as killing sticks, for example, can be accurate up to about 200 m, much farther than a man can throw a stone or spear[3]. ☐

1. Valde-Nowak, P., Nadachowski, A. & Wolsan, M. *Nature* **329**, 436–438 (1987).
2. Luebbers, R.A. *Nature* **253**, 39 (1975).
3. Bell, J. *New Scient.* **99**, 839 (1983).
4. Hess, F. *Antiquity* **47**, 303 (1973).

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Palaeoecology

Chalk grasslands in the ice age

Peter D. Moore

FOR 50 years, studies on the history of the British landscape have shown that there are no habitats that have escaped the influence of human activity, either directly or by the grazing of domestic animals. Indeed, many of the most wild and remote habitats that might have been considered relatively insulated have turned out to be the direct product of human management, including moorland, heathland, blanket mires, many valley mires and montane grassland. Research on the history of the chalk and limestone grasslands of Britain has been hampered by a paucity of evidence, but it is generally assumed that these areas were wooded and that former woodland was cleared by Neolithic or subsequent cultures over the past 5,000 years or so. On page 434 of this issue[1], Bush and Flenley describe the results of pollen analysis of sediments in the Yorkshire wolds, where the chalk exposures of England reach their northernmost limit. The authors show that grassland in this region persisted through the early part of this Flandrian interglacial, 10,000–8,000 years ago.

Chalklands present several problems for the palaeoecologist. The porosity of the substrate means that lakes and peat deposits are scarce or absent, so there are no stratified sediments for fossil analysis. In Britain, the chalk lies in the lowlands of the south and east where precipitation is generally low, so waterlogging and peat formation become even less likely. Spring lines close to the chalk may bear wet woodland, particularly of alder, but when fed by calcareous water and subject to periodic drying and aeration, the preservation of microfossils like pollen grains is generally poor. Similarly, soils contain little pollen, but the lime-rich shells of gastropods do survive and provide valuable information about immediately local habitats. Much of what is known about the history of the English chalk is based on the evidence from fossil snails, both in late-glacial times[2] and during the periods of prehistoric forest clearance[3,4].

Pollen evidence concerning the history of vegetation on the chalk is not wholly lacking, however; information is currently available from the Isle of Wight off the south coast of England and from Sussex in the south of England. Scaife's work on the Isle of Wight[5] shows that the open grasslands of the late-glacial were invaded by juniper and birch woodland soon after 10,000 years before present. The records in Sussex[6,7] do not go back very far and are difficult to interpret because of the abundance of *Alnus* (alder) pollen in the stratigraphy. Because alder is likely to have been a local tree of the valleys, it confuses the picture of vegetation on the upper slopes of the downs.

Bush and Flenley have now discovered a site in Yorkshire where the sediments date back to the end of the last glaciation and the early part of the current (Flandrian) interglacial. Unfortunately, there is a break in deposition after about 8,000 before present and sedimentation commenced again at about 4,300 before present, so the complete Flandrian history of the site remains unknown. But the record of the early part of the Flandrian clearly demonstrates the survival of open grassland right up to the discontinuity. Such persistence of grassland brings to mind the old theories of Wooldridge and Linton[8], who considered chalk grassland to be a natural climax system that had remained free of forest throughout the Flandrian. But the evidence of Bush and Flenley cannot be taken to imply the absence of human influences. Indeed, the erratic behaviour of some trees, such as birch, and the occurrence of some weed species in the pollen rain, lead the authors to propose that Mesolithic people were already disturbing the habitat and maintaining open conditions.

As well as the long-term survival of grassland fragments in general, there is also evidence in these sediments for the specific survival of certain open-habitat species that are taking advantage of disturbances in the forest canopy. The discovery, for example, of *Teucrium botrys* is of particular significance, as this plant has been proposed as a survivor in chalkland refugia. Rose[9] has accounted in this way for its presence on the chalk cliffs at Box Hill in Surrey — a classic refugium site where several relict species are found together. Direct palaeoecological evidence for the persistence of grassland refugia of this sort has previously been restricted to limestone sites in upland regions, such as Malham in Yorkshire, Upper Teesdale and Cwm Idwal in Snowdonia, Wales, but the work of Bush and Flenley adds weight to the hypothesis[10] that many rare and disjunct species in Britain have become restricted in distribution by the spread of postglacial forests.

So, some fragments of Britain's chalk grasslands may date back to the close of the last glaciation and may have offered appropriate conditions for the survival of relict species, but this result does not imply that the grasslands can claim independence from the ubiquitous influence of humans. □

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Palaeoclimatology

Ice core links CO_2 to climate

Eric T. Sundquist

For several years, researchers have struggled to explain the evidence of a 40 – 50 per cent increase in atmospheric CO_2 associated with the end of the last glacial period 10,000 years ago. This evidence, mostly from polar ice cores from Greenland and Antarctica[1], has sorely tested efforts to separate CO_2 and climate into cause and effect. One difficulty has been the short time-span of the ice-core data, which have extended back only 30,000 years and thus did not support conjectures about earlier Pleistocene warm periods. In three papers on pages 403, 408 and 414 of this issue[2-4], a Franco–Soviet collaboration presents ice-core results from the Vostok station in East Antarctica that show a remarkably close association between CO_2 and climate variations extending through the last interglacial period to the preceding glaciation about 160,000 years ago.

In one of these papers, Jouzel *et al.*[2] report the use of the deuterium/hydrogen ratio, measured in the ice at 1-metre intervals throughout the 2,083-metre core, to calculate a continuous record of changes in atmospheric temperatures. They estimate that the temperatures ranged from 9 °C below the present mean annual surface temperature of −55.5 °C (during the most recent and penultimate glacial maxima) up to 2 °C above (during the warmest part of the last interglacial). These conclusions are supported by an analysis of regional isotope-fractionation mechanisms, and by independent estimates from ice-crystal sizes, climate modelling, and temperature-related variations in ice accumulation rates. Even if these temperature estimates become somewhat modified by findings in the future (Jouzel *et al.* estimate the accuracy of the above departures to be about 20 per cent), they appear to be representative of a large portion of the Antarctic continent, and they stand as a powerful constraint for global climate models.

The CO_2 data reported by Barnola *et al.*[3] in another of the papers in this issue not only confirm the deglacial CO_2 increase observed in previous ice-core studies, but also support the inclusion of CO_2 in general theories of Pleistocene climate change. The record shows two dramatic shifts, from about 190 to 280 parts per million by volume, that coincide with the ends of the most recent and the penultimate glacial periods. Between these features, the profile fluctuates between intermediate and low CO_2 concentration, corresponding generally to climatic[5] conditions during the ice ages and interstadial periods (brief warmings within an ice age). Other studies[5] have confirmed, by overlap with the historical record of direct measurements of the atmosphere, that ice cores give a reliable indication of atmospheric CO_2 concentration. The Vostok CO_2 record appears to be irrefutable evidence for a fundamental link between the global climate system and the carbon cycle.

Like the recent less detailed oxygen-isotope record from the same core[6], the Vostok deuterium and CO_2 profiles resemble the shape of the well-known oxygen-isotope curve from deep-sea sediments deposited throughout the world at the same time. (Compare Fig. 2 from Barnola *et al.* on page 410 with curve *c* of Fig. 2 from Jouzel *et al.* on page 404.) The latter is mainly a record of global ice volume. Its spectral characteristics have been shown to support the Milankovitch theory of climate change induced by cyclic variations in the Earth's orbital and rotational configuration with respect to the Sun[7]. Jouzel *et al.*[2] demonstrate that the Vostok deuterium record shows variance density peaks corresponding to periods near 40,000 and 20,000 years, values associated with oscillations in the tilt and precession of the Earth's rotational axis. In contrast, the spectral analysis by Barnola *et al.*[3] of the CO_2 record does not yield a well-defined 40,000-year variance peak. Thus, although both the deuterium and CO_2 records suggest that astronomical forcing is involved, the role of this process is not simple.

Part of the difficulty in relating the Vostok data to other climate records stems from the long-standing problem of correlating ice and deep-sea stratigraphies. Much to their credit, the Vostok investigators do not assume a particular correlation in assigning ages to depths in their core. Instead, they rely on an independent age–depth curve derived from a two-dimensional glaciological accumulation and flow model. This scheme is sparsely documented, and has drawn fire from marine stratigraphers who argue that the Vostok chronology places the onset of the last interglacial period about 10,000 years too early, by comparison with dates assigned to the contemporaneous marine oxygen-isotope stage 5e.

Jouzel *et al.*[2] respond by citing ice-core measurements that support their dating, and point to ambiguities inherent in dating the deep-sea record by correlation with dated reef terraces. A recent analysis of diatom assemblages in a southern ocean sediment core (J. J. Pichon, personal communication) offers the prospect of resolving the correlation problem by matching regional temperature curves. The debate is likely to intensify as a new oxygen-isotope profile, from a uranium-series-dated calcitic vein in the Great Basin in the United States[8], places the beginning of the last interglacial stage even earlier than the Vostok date. These stratigraphic issues have profound implications for determining the timing and mechanisms of global climate change.

The Vostok data will also figure prominently in debate on which processes control atmospheric CO_2 concentration. The Vostok CO_2 data conflict with estimates based on a previous interpretation of marine carbon-isotope records[9]. Nor does the Vostok record confirm the very abrupt CO_2 fluctuations observed in the Greenland ice record[10], although Barnola *et al.*[3] emphasize that their sampling might not be adequate to reveal such changes. Deuterium and CO_2 appear to change almost simultaneously in the Vostok record of warming events, whereas in several cooling events, CO_2 changes lag significantly behind deuterium changes. Barnola *et al.* point out that different mechanisms seem to be required to account for these different modes of climate and CO_2 change. Although they focus on the influence of shallow versus deep modes of ocean circulation change, it is clear that mechanisms involving marine sediments and the terrestrial components of the global carbon cycle should be further considered.

Perhaps the most significant conclusion from the new results is that the global climate system and carbon cycle are intensely interactive. From a statistical comparison of the Vostok temperature profile with linear combinations of the CO_2 profile, Northern and Southern Hemisphere insolation (solar radiation) curves, and several global ice volume profiles, Genthon *et al.*[4], in the third paper in this issue, conclude that CO_2 is a dominant climate forcing factor. This result is not surprising, because it arises essentially from the close resemblance of the Vostok temperature and CO_2 curves. The authors emphasize that their approach does not exclude the reverse influence of climate on atmospheric CO_2.

Most important, Genthon *et al.*[4] express the emerging consensus that CO_2 is an important component in the system of climatic feedbacks that amplify the relatively weak, direct effects of insolation changes. Similarly, carbon-cycle researchers continue to explore the various ways in which atmospheric CO_2 is sensitive to climate change. Further progress requires treating climate and the carbon cycle as parts of the same global system rather than as separate entities. □

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Molecular dynamics

Simulating macroscopic flows

David Heyes

FLUID-FLOW patterns, caused by thermal and pressure gradients, are at the very heart of oceanography, meteorology and rheology (the study of liquids subjected to compressive and shear stresses). On page 427 of this issue, M. Mareschal and E. Kestemont report a numerical simulation of the convection currents caused by thermal gradients. Of particular interest is the authors' demonstration that these currents can occur on a microscopic scale.

Until recently the only way of predicting these flow fields was to postulate the guiding mechanisms of flow, for example, by using equations based on the continuity of mass and momentum. Apart from the numerical problems of solving often 'stiff' equations, these fomulations are simply unrealistic at the high temperature gradients and stress fields that are encountered in, for example, shock studies and non-newtonian liquid flows.

Essentially exact descriptions of flow patterns are nowadays achieved by generating them directly from the highly cooperative motions of individual molecules. This is possible using the computer simulation technique of molecular dynamics. The microscopic foundations of material properties are explored by generating the motions of the individual molecules from the forces that act between them. An assembly of model molecules is placed in a fixed volume, and numerical integration of the equations of motion of the molecules produces a series of fluid structures at time intervals from which the macroscopic properties can be derived. To avoid surface effects, the cell is surrounded by identical images of itself, to give periodic boundary conditions.

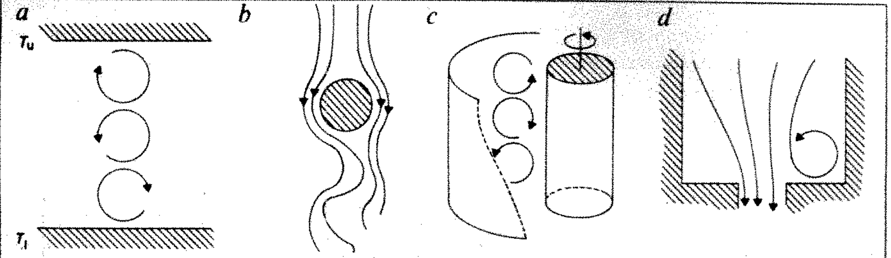
During the 1960s, molecular-dynamic simulations on hard spheres ('snookerball' approximations to molecules) revealed that hydrodynamic flow patterns around a moving test particle could be reproduced by the Navier–Stokes equations, which therefore were shown to be valid on a microscopic distance scale (see, for example, Alder, B.J. & Alley, W.E. *Physics Today* 56–63; January 1984). Recent simulations of fluid flow past an obstruction (Rapaport, D.C. & Clementi, E. *Phys. Rev. Lett.* **57**, 695–698; 1986) and convection currents induced by a temperature gradient (Mareschal and Kestemont in this issue) show that flow patterns such as eddies and thermal rolls (see figure) can be produced in calculations of cells just 30 nanometres across.

These and earlier simulations of more homogeneous fluid flow (see, for example, Ashurst, W.T. & Hoover, W.G. *Phys. Rev.* **A11** 658–678; 1975) reveal that physical properties of liquids remain unchanged when subjected to extreme perturbations. The viscosity, for example, has the same value up to enormous differential flow rates, of the order of thermal velocities across molecular dimensions. Similar microscopically large temperature gradients have an undetectable effect on the thermal conductivity. The macroflow simulations have made use of this 'fortuitous' behaviour to generate statistically significant results. The molecular-dynamics technique is also ideally suited to explore the onset of nonlinear material behaviour, simply by increasing the flow rate or temperature gradient.

Thus, calculations approaching the problem from both ends show that real macroscopic fluid behaviour can occur in systems sufficiently small to be handled by molecular dynamics. Such calculations are normally restricted by the number of individual particles the computer simulation can follow (160,000 in these studies). Both of the recent studies were restricted to two dimensions because of the shortage of computer power. Also they used low values of the Reynolds and Rayleigh numbers (25 and 1,780, respectively) that characterize the stability of a fluid against turbulent flow and convection. These values are comparable to those achieved experimentally because all dimensions, the size of the obstruction and the number of molecules are scaled down drastically in the simulation.

Despite these restrictions, both the studies generate time-dependent flow structures that would have been difficult to obtain with conventional numerical continuum models. If it is possible to use larger Reynolds and Rayleigh numbers, simulations of Taylor vortices (*c* in the figure) and Rayleigh–Bernard convection will be possible, as well as chaotic behaviour and the onset of turbulence.

Such calculations will not be easy, because the distance scale of the flow



Symmetry-breaking fluid flows. *a*, Thermal convection currents between two plates at different temperature ($T_u > T_l$). *b*, Eddy pattern around a cylindrical obstruction. *c*, Taylor vortices in a plane perpendicular to that of rotating central cylinder. *d*, Asymmetrical flow pattern of a non-newtonian fluid undergoing constricted flow.

pattern must be smaller than the smallest dimension of the model cell to avoid distortions caused by the periodic boundary conditions. Therefore, the size of the cell and the number of particles must increase with the Reynolds and Rayleigh numbers. The computer time increases at least in proportion to the number of particles considered, and the real time during which the particles are followed is typically less than 1 nanosecond.

Despite these temporary limitations, molecular dynamics offers the ability, on one level, to generate molecularly based constitutive equations as a basis for macroscopic-flow calculations using finite-difference or cellular-automata algorithms (see Wolfram, S. *Nature* **311**, 419; 1984). In the long term, it could be used to investigate these phenomena directly. ☐

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Nonlinear dynamics

Chaos is good news for physics

Tomas Bohr and Predrag Cvitanović

IN a recent paper, E.G. Gwinn and R.M. Westervelt (*Phys. Rev. Lett.* **59**, 157–160; 1987) explore a route from regular to chaotic behaviour in electronic transport in cooled p-type germanium. The same route to chaos has also been explored by A. Libchaber and co-workers in a very different physical system: the convective flow of mercury (Jensen, M.H. *et al. Phys. Rev. Lett.* **55**, 2798 -2801; 1985). The new experiment is interesting as a study of semiconductor physics and for its many technological ramifications; but both experiments illustrate the high precision with which experimentalists can now test the theory of transitions to chaos.

Chaos has become a cover name for an active branch of physics, describing the highly irregular, unpredictable behaviour that occurs in most deterministic, nonlinear dynamical systems. Many mathematical concepts dating back to the last century — and, until recently, regarded by physicists as irrelevant to the description of natural phenomena — have suddenly become important. Notions such as the 'Hausdorff dimension' and 'fractals' have replaced Euclid's dimensions and straight lines, because schoolbook geometry is of little use when confronted with the bewildering complexity generated by nonlinear dynamical systems. The work of B.B. Mandelbrot and especially his book *The Fractal Geometry of Nature* (Freeman, New York, 1982) has been instrumental in turning physicists' attention to such fractal structures and introducing the relevant mathematical concepts.

A decade ago, M.J. Feigenbaum (*J. stat. Phys.* **21**, 669–706; 1979) conjectured that many physical systems should make the transition from regular (periodic) to chaotic motion in a qualitatively and quantitatively universal fashion, through sequences of period doublings. His approach was very unconventional: instead of modelling a realistic dynamical system he used a computer to iterate a simple map. The regime where chaos occurs is quite inaccessible by standard approximation techniques; but the map reduces the problem to its essence, making it possible to follow the strongly nonlinear behaviour with a minimum of effort and still extract universal quantities. The theory has been confirmed experimentally in many physical systems, ranging from convective flows in liquids to cardiac arrhythmias in chicken hearts. Original articles are collected in two reprint collections: Hao Bai-Lin (ed.), *Chaos* (World Scientific, Singapore, 1984); and *Universality in Chaos* (Hilger, Bristol, 1984; edited by P.C.).

In their experiment, Gwinn and Westervelt apply a time-varying voltage to a crystal of p-type germanium and record the current passing through it. The system has an oscillatory instability so that even a static (d.c.) voltage larger than some threshold value induces a periodic oscillation of the current, with an extremely stable frequency. When the additional time-varying component is added to the voltage, the two frequencies compete, and if the amplitude of the driving frequency is large enough, chaotic behaviour develops. The interaction of pairs of frequencies is of considerable theoretical interest because of the generality of the phenomenon. As the energy input into a dissipative dynamical system is increased, typically first one and then another intrinsic mode of the system is excited, and their interaction can give rise to chaos. Competing modes usually give rise to mode-lockings: the frequencies adjust slightly to fall into step, making their ratio a rational number. In that case the response is periodic; an irrational ratio corresponds to quasiperiodic behaviour — the motion never quite repeats itself.

The aim of the experiment was to test the theory of the transition from quasiperiodicity to chaos. Mode-lockings (which have interesting properties of their own) are avoided by tuning the external frequency so that its ratio to the internal frequency equalled the golden mean, $(\sqrt{5}-1)/2$. The choice of this ratio does not represent a return to mediaeval alchemy, but is dictated by number theory: the golden mean belongs to a family of irrational numbers for which it is hardest to give good rational approximants. As experimental measurements have limited accuracy, physicists usually do not expect number-theoretic subtleties, such as how irrational a number is, to be of any physical interest. In the theory of transitions to chaos, however, the starting point is the enumeration of asymptotic motions of a dynamical system, and it is through this enumeration that number theory enters and comes to have a central role.

The output of the experiment is the time variation of the current, the full record of which contains much superfluous

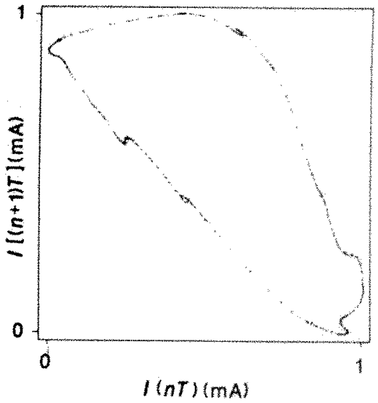

Fig. 1 The strange attractor at the onset of chaos. The current (a constant current of 5 mA has been deducted) is plotted for each cycle of the external drive. T and n, respectively, are the period and number of drive cycles and the current at cycle $n+1$ is plotted against the value at cycle n. (From Gwinn, E.G. & Westervelt, R.M. *Phys. Rev. Lett.* **59**, 157–160; 1987.)

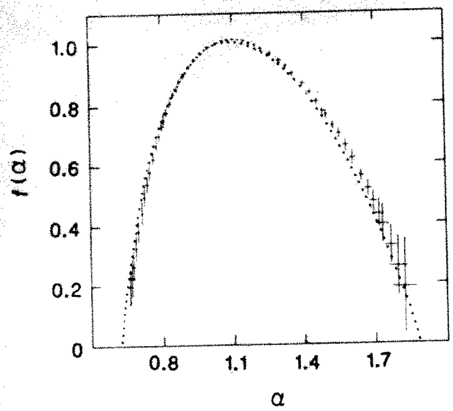

Fig. 2 The function of $f(\alpha)$ quantifying how often a given scaling index or pointwise dimension occurs on the attractor. The error bars indicate the standard deviation of the mean of three data sets. The universal circle-map prediction is shown as a dotted curve. (From Gwinn, E.G. & Westervelt, R.M. *Phys. Rev. Lett.* **59**, 157–160; 1987.)

information. Previously, the results would have been presented as a frequency power spectrum. Today the data (50,000 data points would be typical) are manipulated in the way that a theorist analyses a numerical simulation. First, the system is reduced from a continuous time recording to a discrete series of stroboscopic flashes. This method, devised a century ago by H. Poincaré, makes it possible to survey the dynamics visually. The experimental Poincaré map is shown in Fig. 1. The value of the current at each period of the external drive is plotted against its value at the next period. (The first hundred or so cycles, the transients, are not plotted.) The fact that the response is quasiperiodic implies that the dots on the figure would eventually fill up some closed curve. If the transients had been shown on Fig. 1, we would see points initially far away rapidly approaching that curve, which is therefore an 'attractor'. Precisely at the onset of chaos — which was reached in the experiment by varying the amplitude of the drive — the kinky structure visible on the figure emerges. It is called a 'strange attractor' because of the unusual way points are distributed on it and this distribution contains information about universal features of the transition to chaos.

The strangeness of the attractor can be probed by looking at the distribution of points around some reference point. For a reference point, P, on the attractor we define $N_P(r)$ as the number of neighbours within distance r along the attractor. On a usual (non-strange) quasiperiodic attractor, the points are distributed smoothly; a segment of the attractor looks like a line. Thus, $N_P(r)$ scales with r as $N_P(r) \propto r$ for any point P. For the strange attractor in the figure this is not necessarily true. If we pick a point at random, the theory predicts $N_P(r) \propto r^\alpha$ with α varying between 0.6326 . . . and 1.8980 . . ., and the point P is said to have the pointwise dimension α.

A powerful formalism for confronting the experiment with the predictions of the theory was developed recently by T.C. Halsey *et al.* (*Phys. Rev.* **A33**, 1141–1151; 1986). They look at the attractor as a superposition of 'subfractals'; namely, for each α, the set of points with pointwise dimension α. A function $f(\alpha)$ is introduced to quantify how often a given value α appears on the attractor — more precisely, $f(\alpha)$ is the Hausdorff dimension of the set of points with pointwise dimension α. Figure 2 shows this curve for the theory and the experiment. The dots are the theoretical prediction computed by iterating a so-called circle-map. The crosses represent $f(\alpha)$ calculated from the experimental attractor shown in Fig. 1. Given that there are no adjustable parameters, the agreement between the theory and the experiment is remarkable. (The error bars at the edges of the α-interval come from the sparseness of points contributing in those limits; here the finiteness of the data sets and noise play a large part.)

Theory and experiment thus interact in a fruitful way, and it is fascinating that the fractal properties of strange attractors can be measured with such precision in physical systems. Theoretically the most striking fact, not anticipated until a decade ago, is that the dynamical systems approaching chaos do so in a universal fashion. Other fields of physics, notably those concerned with growth or aggregation, reveal all kinds of fractal behaviour, but it is not yet clear what kind of universality (if any) to expect there. ☐

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Intermediate filaments

Looking for a function

Benjamin Geiger

STUDIES on the molecular properties of the cytoskeleton are largely motivated by the desire to understand the functions of these cytoplasmic filaments in cells and tissues. Physiological studies, morphological observations and biochemical characterization of proteins in the cytoskeleton have allowed molecular models, albeit tentative, of the possible cellular activities in which the various classes of filaments take part. These models, despite their tendency to be oversimplified, have contributed a great deal to current concepts of mechanisms of cell motility, mitosis, transcellular transport, adhesion, modulation of membrane activity and cellular morphogenisis. Now, however, experimental work[1-4] is providing a test for many of these ideas about the physiological functions of intermediate filaments, one class of cytoskeletal filaments.

Until recently, most information has come from the other two classes of cytoskeletal filaments, microfilaments and microtubules, which, together with batteries of associated proteins, have been extensively characterized. Moreover, the availability of excellent and well-studied model systems such as the contractile unit of skeletal muscle or the interaction of dynein with microtubules in cilia and flagellae have provided clues about the cytoplasmic activities in which actin and tubulin are involved.

The structure–function relationships of the third cytoskeletal network, the intermediate filaments, are less well characterized. Despite the fact that the primary structure of many intermediate-filament subunits is known and their cellular distribution extensively documented, only limited molecular information has so far been available on their behaviour *in vivo*. In the absence of specific data, it has been suggested that intermediate filaments are involved in mechanical integration of cytoplasmic space[5] or in a skeletal framework of the cytomatrix (see refs. 6,7 for reviews). Well-controlled experiments relating their molecular properties to specific cytoplasmic events are, however, still needed to understand their functions in more detail.

Inagaki *et al.*[1] recently investigated specific phosphorylation events in modulating the assembly of vimentin molecules into intermediate filaments. These authors show that vimentin is an excellent *in vitro* substrate for protein kinase C and cyclic AMP-dependent protein kinase, but not of several other kinases. Moreover, phosphorylation by the cyclic-AMP-dependent kinase induces the dramatic disassembly of vimentin filaments. Analysis of tryptic phosphopeptide maps indicates that the sites of phosphorylation with the two kinases are distinct, and the authors suggest that a single, site-specific phosphorylation of vimentin (as well as its

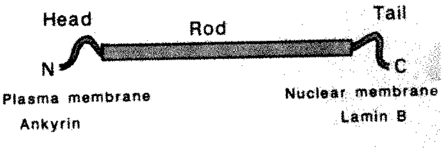

Fig. 1 Intermediate-filament subunit (vimentin or desmin) presenting binding sites for plasma-membrane-associated ankyrin at the amino-terminal head domain, and binding sites for lamin B at the carboxy-terminal tail region.

dephosphorylation) is important in the modulation of filament structure and organization.

These new findings could be related to earlier observations[5-10] which correlated changes in intermediate-filament structure in cells with phosphorylation events. The phosphorylation-dependent disassembly of intermediate filaments may produce a molecular solution for a cellular dilemma: how do dynamic cellular processes such as cytokinesis or changes in cell shape occur in the presence of the notoriously insoluble network of intermediate filaments? Data obtained using the immunofluorescence and electron microscopes indicate that cells can transiently dissociate their intermediate filaments and pack their subunits into non-filamentous spheroidal structures[11-13]. It is not clear whether a site-specific phosphorylation-dependent dissociation similar to the one documented by Inagaki *et al.*[1] is involved, or whether other modifications are effective during the dynamic rearrangement of the cytoplasm *in vivo*. To elucidate this question, it is necessary to define and localize the critical phosphorylation site along the vimentin molecule and to determine directly if specific, spatially controlled phosphorylation–dephosphorylation events are indeed involved in the physiological modulation of filament integrity and structure.

Another related matter concerns the native interactions of vimentin or other intermediate filaments with various cytoplasmic organelles. Many previous studies, based on immunocytochemistry and localization in the electron microscope, pointed to an apparent association of intermediate filaments with many cellular structures. It had been claimed that in addition to their well-documented interactions with desmosomes, intermediate filaments are often detected in close association with the nucleus, the plasma membrane, dense bodies of smooth muscle, microtubules, fat globules of adipocytes and other elements (see ref. 6 for a review). These observations stimulated much speculation concerning the roles of intermediate filaments as specific cytoplasmic organizers, yet the basis for the presumptive interactions remained obscure.

In a series of recent biochemical studies, Georgatos and Blobel[2,3] show that purified vimentin binds to different fractions of avian erythrocyte membranes through two distinct domains. Sites located at the carboxy-terminal tail of the vimentin molecule bind specifically to nuclear envelopes in a cooperative fashion, whereas the plasma-membrane fraction interacts in a saturable manner with the amino-terminal head of the vimentin molecule. The central rod segment of vimentin has no apparent affinity to either fraction (see Fig. 1). Binding studies reveal specific vimentin-binding constituents along these two types of membrane. It is shown that major nuclear binding sites for vimentin are lamin B or the lamin A–lamin B hetero-oligomers. Interestingly, lamins A and C, which associate with lamin B to form the nuclear lamina at the endofacial surfaces of the nuclear membrane, have a similar primary and secondary structure to intermediate filaments[14,15]. Other new work[4] shows that the membrane-skeleton protein ankyrin could provide a second type of binding site to the amino-terminal head region of vimentin or of desmin, a related intermediate-filament protein of muscle cells (Fig. 1).

The specific interaction of vementin, and possibly other intermediate-filament subunits with lamin B, raises an interesting problem. In their heteropolymeric form, lamins are known to be confined to the endofacial surfaces of the nuclear envelope, whereas intermediate filaments are located throughout the cytoplasm and outside the nucleus. Thus, physical interaction between vimentin and lamins (if and when it takes place *in vivo*) could occur through the nuclear pores, where elements of the nuclear lamina are exposed to the cytoplasm. This suggestion of a direct and specific interaction between elements of the cytoplasmic matrix and peripheral elements of the nuclear matrix is not new. Ultrastructural studies carried out several years ago suggested that such interactions exist and take place through the nuclear-pore complexes[16-20]. The identification of another binding system along the plasma membrane provides a possible mechanism for nucleolemmal/plasmalemmal anchorage and communication (see scheme in Fig. 2). The new *in vitro* results[1-4] seem to complement the existing data concerning the molecular basis and possible mechanisms underlying the physiological interactions of intermediate filaments.

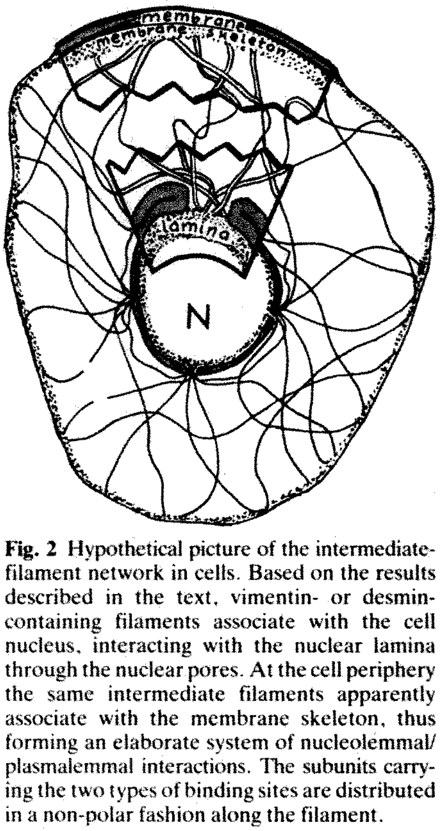

Fig. 2 Hypothetical picture of the intermediate-filament network in cells. Based on the results described in the text, vimentin- or desmin-containing filaments associate with the cell nucleus, interacting with the nuclear lamina through the nuclear pores. At the cell periphery the same intermediate filaments apparently associate with the membrane skeleton, thus forming an elaborate system of nucleolemmal/plasmalemmal interactions. The subunits carrying the two types of binding sites are distributed in a non-polar fashion along the filament.

It is too early to evaluate the functional significance of these new observations, yet specific questions and working hypotheses can now be put forward relating the new molecular and structural findings to specific cytoplasmic and nuclear events. Does the nuclear lamina that is exposed at the nuclear pores act as an organizing centre for intermediate-filament assembly? Is ankyrin (or a homologous molecule in non-erythroid cells) a physiological linker of intermediate filaments to the plasma membrane? Are the interactions of vimentin with the nucleus or with the plasma membrane modulated by post-translational modifications of either of the molecules involved? Do the mechanical interconnections between the nucleus and the membrane play a general role in the spatial organization of the cytoplasmic matrix or in the active or passive transport of macromolecules between the nucleoplasmic and cytoplasmic compartments? Are there other intermediate filament-associated proteins on different cellular organelles? How are the site-directed phosphorylations (or dephosphorylations) of vimentin spatially and temporally regulated? Do other intermediate filaments, such as keratins, neurofilaments or glial filaments, have binding properties similar to those of vimentin and desmin, and are they comparably affected by specific phosphorylations? The tools are now ready for concerted efforts to answer these and other questions about the organization and dynamics of the cytoplasm. ☐

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High-energy physics

Mixed beauty at Hamburg

David J. Miller

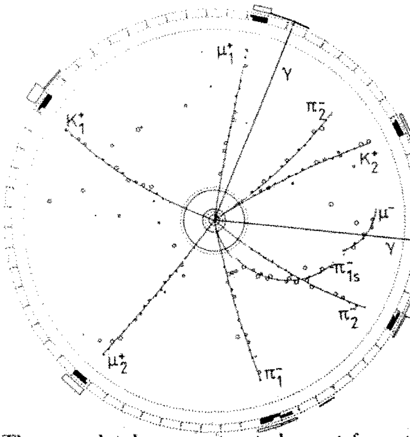
AMONG the many exciting developments in particle physics reported at a recent meeting*, the most interesting came from the host organization, DESY (Deutsches Elektronen-Synchrotron). The ARGUS collaboration there has seen positive evidence of the 'mixing' of a meson, containing a beauty quark, with its antiparticle. ARGUS has also found a surprisingly large rate for the production of baryons containing beauty quarks. The standard model of particle physics survives intact for another year, having passed several new tests with no serious contradiction of its ingredients: electroweak theory, quantum chromodynamics and the number of quark 'generations'. S.L. Glashow (Harvard) suggested three alternative "fables" of what might emerge at the next generation of accelerators.

Particle–antiparticle mixing has been studied for more than 20 years with K^0 mesons. These particles consist of a down quark and a strange antiquark, $(d\bar{s})$, but when they decay they sometimes appear to have changed into the $\bar{K}^0$ antiparticle $(\bar{d}s)$. In their decays they behave like two orthogonal linear combinations of K^0 and $\bar{K}^0$, called K-long and K-short because of their different lifetimes. An early success of the standard model was to explain K^0–$\bar{K}^0$ mixing at the same time as predicting the charmed quark. Now the ARGUS group (W. Schmidt-Parzefall, DESY) has seen three distinct independent signals for B^0–$\bar{B}^0$ mixing ($\bar{B}^0$ is a meson with down antiquark and beauty quark $(\bar{d}b)$; B^0 is $d\bar{b}$).

In both B^0 and K^0 decays, the identity of the decaying quark (particle or antiparticle) is most clearly defined when it decays to a charged lepton (either an electron or a muon), accompanied by a lighter quark and a neutrino (or antineutrino). This is similar to nuclear β-decay, in which an electron (e^-) and an antineutrino are produced when a down quark in a neutron (udd) turns into an up quark in a proton (uud). Down, strange and beauty quarks, all being negative, decay to give a negative electron or muon (μ^-), but their antiquarks give a positive lepton (e^+, μ^+).

The ARGUS group produced pairs of B^0 and $\bar{B}^0$ in e^+–e^- collisions at an energy where no extra particles can be produced. In the absence of mixing, one would expect the decay particles to include pairs of leptons of unlike-sign (one from the particle, the other from the antiparticle). But in one sample the group saw 5 like-sign lepton pairs and 23 with unlike-sign. The group also achieved a rare success: a fully reconstructed event in which enough tracks could be observed to give a unique identification (see figure). Both decaying particles were B^0, though one of them must have been produced as $\bar{B}^0$. The UA1 collaboration at CERN (quoted by Schmidt-Parzefall) also reports a like-sign dimuon signal that can be interpreted as B^0–$\bar{B}^0$ mixing, but not as clearly.

These results put tight constraints on some of the arbitrary parameters of the standard model; in particular on the parameters of the unitary matrix (the Cabibbo–Kobayashi–Maskawa matrix) that describes the weak couplings of all the quarks. The matrix can still be kept unitary (M. Schifman, Institute of Theoretical and Experimental Physics, Moscow; A. Sirlin, New York University), which is necessary if the theory is to conserve the overall sum of quarks and antiquarks, but only if another free parameter of the model is constrained: the mass of the top quark would have to be at least 50 GeV, contradicting the CERN claim (now retracted) to have discovered top between 30 and 50 GeV at UA1 (see *Nature* **311**, 210; 1984). This puts the threshold for $t\bar{t}$ production out of range of the Stanford Linear Collider now tuning up, but within range of Large Electron–Positron project (LEP) phase 2 at CERN.



(P. Söding, DESY)

The completely reconstructed event from the ARGUS experiment in which B^0–$\bar{B}^0$ mixing was identified. A B^0–$\bar{B}^0$ pair is created by an electron–positron collision. The antiparticle turns into a particle by mixing. The two particles then undergo a series of decays to emit the detected particles μ_1^+, π_1^-, π_1^-, K_1^+ in the first case and μ_2^+, π_2^-, π_2^-, K_2^+ and two photons γ in the second. The open circles mark the detected track and the solid lines the computed track.

Researchers at CERN have made an improved measurement of the violation of 'CP' symmetry, in which the behaviour of a particle is indistinguishable from that of its antiparticle, as seen in a mirror. The spontaneous breaking of this symmetry (first observed in K-meson decays in 1964) may be a clue to the physics of the cosmological Big Bang (reviewed by M.S. Turner, Chicago University). The parameter in the Cabibbo–Kobayashi–Maskawa matrix that describes CP violation should cause a small difference between the decay rates of K-short mesons to two charged or two neutral pions; a difference that was confirmed for the first time in the CERN experiment (I. Mannelli, University of Pisa).

In principle, CP violation should be observed in B-meson decays, and its rate can be predicted as the mixing parameters are known. Present accelerators, however, would take 100 years to supply the data to measure the effect (Schmidt-Parzefall) and a new generation of medium-energy, high-luminosity, electron–positron colliders is proposed to complement the high-energy ones being constructed now (described recently in News and Views: *Nature* **329**, 107; 1987).

The newest result reported at the meeting is also the hardest to fit into the standard model (Schifman). The ARGUS collaboration has measured an unexpectedly high rate for the production of pairs of beauty baryon and beauty antibaryon. (Beauty baryons contain a beauty quark and two light quarks; bud, for example.) But there is much work to be done, both experimental and theoretical, before this can be taken seriously. Schifman spent some time explaining how the intrinsic complexity of the "kitchen of strong interactions" destroys some of the simple predictions of pure electroweak theory, and may affect beauty baryon production.

Oddly enough, a similar concept of Schifman's kitchen, but called 'weather' (also denoting nonlinear fluctuations at an unobservably small scale) was introduced by D. Schramm (University of Chicago) to explain disagreements between the standard model of supernovae and the optical observations of the recent supernova 1987A. The neutrino signals are thought to come through the weather like radar through fog, and agree very well with models of the simple primary process. Glashow's three fables, each a minimal alteration of the standard model within present experimental constraints, were presented as examples of the new physics (heavy decaying neutrinos; extra Higgs particles; 'chiral colour' giving a new particle zoo) that could emerge at LEP, or the proposed Superconducting Supercollider in the United States. Although explaining some features of the standard model, the fables avoid any reference to such ambitious (and experimentally unsupported) schemes as technicolour, supersymmetry or superstrings. ☐

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* International Symposium on Lepton and Photon Interactions at High Energy, 27–31 July 1987, Hamburg, FRG

Neurobiology

Taking apart NMDA receptors

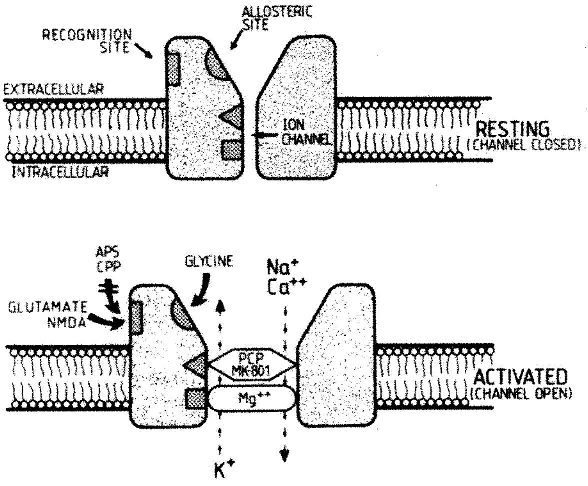
Alan C. Foster and Graham E. Fagg

If the frequency of publications in *Nature* is an indication of the frontiers of scientific research, then excitatory amino-acid receptors, and specifically, the NMDA (*N*-methyl-D-aspartate) receptor, must rank as one of the 'hottest' topics in neurobiology at the moment. Why is this? In a large part, it is because the NMDA receptor — one of the classes of glutamate receptor mediating excitatory synaptic transmission — is being found to play a critical role in many complex neurophysiological and 'plastic' events which underlie central neural development and function in vertebrates. Recent work shows that NMDA receptors are involved in long-term potentiation, burst firing and the generation of patterned activity in neuronal networks; in use-dependent stabilization of synaptic connectivity in young animals; in some forms of learning in mature animals; and in pathologies such as epilepsy, spasticity and ischaemic neuronal death (see refs 1, 2 for reviews).

What do we know about the molecular organization and structure of the NMDA receptor? Can its contribution to brain physiology and pathology be rationalized in terms of the receptor channel complex? Although precise correlations of structure and function await the application of molecular-biological techniques, data now emerging from electrophysiological and biochemical investigations show that the NMDA receptor comprises distinct domains (see figure) whose interactions form the basis of the initiation and regulation of receptor activity. The NMDA receptor complex is not simply a ligand-gated ion channel (as are many fast-acting transmitter receptors), but is subject to additional regulation by the simple amino acid glycine and, uniquely, by voltage-dependent binding of magnesium ions inside the channel. Yet another binding site for psychoactive drugs such as phencyclidine (PCP) and MK–801 is also located within the channel.

What is known about the domains in the NMDA receptor complex? The transmitter recognition site is the most extensively characterized pharmacologically. Here, L-glutamate and L-aspartate bind with high affinity, whereas analogues of dicarboxylic amino acid such as AP5 (2-amino-5-phosphonopentanoate) and CPP (3-[2-carboxypiperazin-4-yl] propyl-1-phosphonate) are the most potent and widely used competitive antagonists[3]. There are hints from recent structure-activity studies, however, that such antagonists bind partly to another attachment point close to the transmitter-recognition site and that the charge at the ω-terminal is a determinant of agonist versus antagonist properties[4].

The most significant discovery for understanding the channel domain of the NMDA receptor was that the agonist-evoked conductance is blocked in a voltage-dependent manner by magnesium ions[5,6]. Single-channel experiments show that this unusual form of voltage-gating largely results from blockade of the open channel by Mg^{2+}, probably at a site deep in the channel, near to the intracellular face of the membrane. At membrane potentials close to resting and at normal extracellular Mg^{2+} concentrations (1–2 mM), the channel is blocked and agonists induce little current flow. As the membrane is depolarized, the block is relieved, probably because the decreased transmembrane electric field less effectively 'pulls' Mg^{2+} into the channel. The result of this voltage-dependent channel block is that the NMDA receptor conductance exhibits regenerative properties which, together with Ca^{2+}- or depolarization-triggered hyperpolarizations, underline its involvement in phenomena such as rhythmic firing and epileptiform activity.



Model of the NMDA receptor channel complex. Top, closed; bottom, open. The receptor appears to be proteinaceous[4] and may consist of multiple subunits. Four sites in the complex have been identified on the basis of their function: (1) the transmitter recognition site, with which agonists (NMDA, L-glutamate) and competitive antagonists (AP5, CPP) interact; (2) a site through which glycine allosterically up-regulates receptor function, possibly by facilitating agonist-induced transitions to an open-channel configuration; (3) a cation-binding site inside the channel, where (depending on membrane potential) Mg^{2+} can bind and block transmembrane ion fluxes; and (4) a PCP-binding site, which requires agonist activation at the transmitter recognition site, interacts with the divalent cation binding site, and at which psychoactive drugs such as PCP and MK–801 bind to function as open-channel blockers.

Recently, several organic molecules (such as PCP, ketamine, MK–801), all of which interact with 'PCP-binding sites' in brain membranes, have been shown to antagonize non-competitively NMDA receptor responses. The blocking actions of these substances are dependent on membrane voltage (like Mg^{2+}) and on activation of the receptor with an agonist such as NMDA (use-dependent)[8,9]. Such use-dependence is also apparent in radioligand binding experiments[4,9]. Very recent work shows that recovery from blockade by ketamine is also dependent on agonist application, suggesting that this substance can become 'trapped' inside the channel, and an analysis of the voltage-dependence of the block indicates that ketamine interacts with a site roughly midway across the membrane field[10]. These data are consistent with the hypothesis that PCP and related compounds block NMDA receptor responses by binding inside the open channel and impeding trans-membrane ion fluxes.

Do PCP and MK–801 interact with the same site as magnesium ions? Divalent cations interact with the binding site labelled by [^{3}H]thienyl-PCP (TCP) and [^{3}H]MK–801 in a complex manner, causing both enhancement and inhibition of binding at appropriate concentrations[11,12]. Whereas all divalent cations tested to date inhibit binding, only those possessing NMDA-antagonist properties enhance binding, suggesting that this latter effect represents an interaction within the NMDA-receptor ion channel and that PCP/MK–801 and Mg^{2+} bind to separate sites within this domain.

The discovery of a third domain within the NMDA receptor complex, through which glycine regulates receptor activity, began with observations that this simple amino acid markedly augments NMDA-evoked responses in cultured neurons[13]. This effect is strychnine-insensitive, and results principally from an increase in the frequency of NMDA-elicited channel opening. The possibility that glycine is a specific regulator of NMDA-receptor mechanisms has rapidly gained credence from the similar regional distributions of strychnine-insensitive glycine binding sites and NMDA receptors in the brain[14] and, more directly, from very recent findings that the stimulation of [3H]TCP and [^{3}H]MK–801 binding by NMDA-receptor agonists is potentiated by this amino acid[4,11,15]. Although the details of the interactions between receptor domains remain to be elucidated, these data suggest the fascinating hypothesis that, in contrast to its inhibitory function in spinal tissue, glycine acts in higher brain areas to facilitate allosterically NMDA receptor-mediated excitation.

The domain structure of the NMDA receptor (see figure) is, in many respects, similar to that which has been described for other transmitter-gated ion channels. At both the nicotinic acetylcholine and $GABA_A$ receptors, 'non-competitive' antagonists interact with the receptor complex in an agonist-dependent manner, reflecting, as in the case of PCP or MK-801 at the NMDA receptor, a preference for activated or open states of the ion channel. At the $GABA_A$ receptor, the well-documented ability of benzodiazepines to enhance receptor activity allosterically can be compared with the action of glycine at the NMDA receptor. As well as these common features, the NMDA receptor has evolved a unique form of voltage-dependent gating, involving binding of Mg^{2+} inside the channel. Hence, 'switching on' NMDA receptor mechanisms requires at the minimum: (1) binding of transmitter to its recognition site; and (2) relief of the channel blockade (for example, by local depolarization of the postsynaptic membrane by other excitatory inputs). In addition, receptor function may be subject to rapid up- or down-regulation by changes in the levels of glycine and/or Mg^{2+} in the local extracellular environment.

Now that the basic functional organization of the NMDA receptor is understood, what are the next steps? First, understanding the full physiological implications and intricacies of its regulatory mechanisms requires detailed studies of the interactions between receptor domains and their stoichiometric relationships, the modulation of different affinity states, and the seemingly diverse effects of divalent cations. In view of recent reports that Zn^{2+} non-competitively antagonizes NMDA-evoked responses at a site distinct from that at which Mg^{2+} binds [16,17], one might ask how many more domains of potential significance there are. Second, although the biophysical properties of the NMDA receptor-channel complex can account for its involvement in electrical phenomena such as signal amplification and rhythmic firing, they cannot alone explain its role in prolonged events like long-term potentiation or synaptic reorganization. In this respect, a clue for future studies is that the NMDA receptor channel is permeable to Ca^{2+} (ref. 18), which may activate long-term biochemical changes in the post-synaptic neuron.

The goal of the reductionist approach is to understand function in terms of molecular structure. Such studies are under way for the nicotinic acetylcholine receptor[19] and, given its unique properties, these will undoubtedly be of special interest for the NMDA-receptor complex. The path ahead is certainly long, but the recent work described here is a valuable initial step towards elucidating the molecular basis of some of the most intriguing excitatory and plastic events in the central nervous system. ☐

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John Howard Northrop (1891–1987)

John Northrop probably did more than any other individual to establish the view that pure enzymes are indeed proteins. He was not, however, the first to crystallize an enzyme. That honour belonged to J.B. Sumner of Cornell University, who reported the crystallization of urease from jack bean in 1926. Northrop's work, however, was both more extensive and more searchingly critical than Sumner's. Both men had to face widespread scepticism concerning their work: Richard Willstätter in Munich, with his great prestige, had denied the protein nature of enzymes. Most biochemists, particularly in Europe, followed Willstätter, and for years discounted the evidence of Sumner and Northrop.

Northrop, born in Yonkers, New York, did both his undergraduate and graduate work at Columbia University, receiving his PhD in 1915. In 1916 he became an assistant in the laboratory of Jacques Loeb at the Rockefeller Institute in New York. Loeb was a major influence in his development. From the beginning Northrop worked on enzymes; many of his early papers concerned the kinetics of enzyme-catalysed reactions. After Loeb's death in 1924, Northrop arranged to be transferred to the Princeton branch of the Rockefeller Institute. He loved open country, and strongly disliked living in the city.

It was in Princeton that he achieved the crystallization of three major proteolytic enzymes — pepsin in 1930, and trypsin and chymotrypsin a few years later — and provided the most rigorous test then available to demonstrate that they are pure proteins. He well knew that the preparation of a substance in crystalline form is not in itself an adequate criterion of purity; mixed crystals of two or more closely related substances were a common occurrence. Northrop's test for purity involved careful solubility studies in well-defined media, with application of the phase rule of Willard Gibbs. A truly pure protein in such a medium should give a constant solubility, independent of the amount of crystalline phase in equilibrium with the solution. He was able to show, for his preparations, that this was indeed the case. Sumner, in his pioneer work, had not applied such rigorous tests; so on the whole it was Northrop's work that was most influential in gradually convincing the sceptics.

Northrop's associate, Moses Kunitz, shared much of this work, and later continued independently to crystallize other enzymes, notably ribonucleases and deoxyribonucleases. Northrop and Kunitz, together with Roger Herriott, described this research in their book *Crystalline Enzymes*.

In 1938, the trustees of the Rockefeller Institute closed the Princeton branch of the Institute. Most of the Princeton workers moved back to New York or retired. Northrop, however, refused to live in New York and the trustees permitted him to move elsewhere, to a place of his choice, while still remaining a member of the Institute and receiving its support for his work. In 1939 he settled at the University of California at Berkeley, where he became a professor and did extensive work on bacteriophage, his approach being similar to that of his earlier work on enzymes. But he missed the biological significance of the life cycle of bacteriophage, and his findings were eclipsed by the work of molecular biologists.

In 1946, Sumner and Northrop received the Nobel prize in chemistry (together with Wendell Stanley, for his work on viruses). At last the sceptics had been convinced of the validity of Northrop and Sumner's work. This was the chief of the many honours that Northrop received. He retired from Berkeley in 1959, and characteristically chose to live far away from noise and bustle, in the rather remote district of Wickenburg, Arizona, where he died.

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When evolution cannot go into reverse

SIR—P.L. Williams (*Nature* **328**, 21–22; 1987) incorrectly refers to "ambiguity" in our article on irreversible evolution (*Nature* **326**, 128; 1987). For the purpose of our discussion, the process was defined as involving "evolutionary routes that, once taken, preclude return", and illustrated with an example taken from hymenopteran wasps in which both biology and theory are understood. In none of the cases cited by Williams is there any reason to believe that a strict retracing of evolution could not occur if all ancestral environments were retraced. In the peppered moth example, the complications cited by Williams do not prevent reversible evolution. The whole point of our article was that, on occasion, there are good reasons why an evolutionary route would not be retraced even given the ancestral intermediate environments.

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Function of a new globin gene

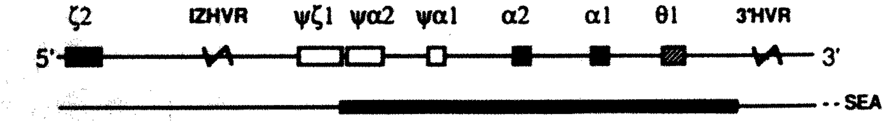
SIR—Recently a new gene ($\theta 1$) has been identified at the 3′ end of the α-globin cluster in several species[1-5] including man. It has been suggested that this gene may be functional in higher primates because in the orang-utan and olive baboon it has all of the structural features necessary for expression and in man it has given rise to a processed pseudogene elsewhere in the genome[6]. Further evidence for its functional status comes from comparisons of the silent versus replacement site substitutions in two primate $\theta 1$ genes which indicate that it has been evolving under selective constraints[2]. Speculation about the function of $\theta 1$ has centred on its possible role as an embryonic globin involved in placentation[1,2].

At present no protein or messenger RNA corresponding to the $\theta 1$ gene has been reported. In the past, the function of various globin genes has been established by identifying haemoglobins corresponding to their gene products and observing the effect of naturally occurring mutants of globin synthesis (thalassaemias) on the phenotype of affected individuals. As far as we know, however, there are no unassigned human globins and therefore no candidate gene products for $\theta 1$. Interestingly, analysis of the predicted amino-acid sequence of the $\theta 1$ counterpart in horse suggests that it may not encode a viable globin-like protein at all (see page 465 of this issue[7]) but as it has clearly been conserved throughout evolution, may subserve some other function.

In man, the $\theta 1$ gene lies at the 3′ end of the α-globin complex which includes an embryonic gene (ζ), two adult α genes ($\alpha 2$ and $\alpha 1$) and three pseudogenes ($\psi\zeta 1$, $\psi\alpha 1$ and $\psi\alpha 2$) (see figure). We have recently shown that a determinant for α thalassaemia in South-East Asia results from a deletion that removes both α genes ($\alpha 2$ and $\alpha 1$) and the $\theta 1$ gene (see figure)[8]. Heterozygotes for this determinant have a mild anaemia but are otherwise normal. Homozygotes survive *in utero* until 28–32 weeks of gestation but, with rare exceptions[9], die in the perinatal period due to the effects of prolonged intrauterine hypoxia; this condition is known as the Hb Bart's hydrops fetalis syndrome. The fact that such infants can survive at all until this late stage in development makes it very unlikely that the $\theta 1$ gene encodes a critical embryonic globin.

Observations on homozygotes for this deletion may also be instructive in evaluating other possible roles for the $\theta 1$ gene. Although infants with the Hb Bart's hydrops fetalis syndrome have a relatively high incidence of congenital malformations, there is no consistently abnormal developmental pattern and it is likely that such abnormalities are secondary to chronic intrauterine hypoxia. We have re-evaluated the genotype of a child with this syndrome who has been intensively treated and kept alive by blood transfusions[9]. We find this child to be homozygous for the South-East Asian α-thalassaemia determinant, both $\theta 1$ genes are deleted (see figure), and yet the child appears to have developed normally, indicating that $\theta 1$ does not encode any other critical protein that cannot be replaced by regular blood transfusion.

It is possible that $\theta 1$ is only one member of a family of θ-like genes[1] and that deletion of a single member ($\theta 1$) is of little or no consequence. However, the observations reported here raise some doubts as to whether $\theta 1$ is expressed as a protein; it is unlikely to be a part of a viable haemoglobin molecule[7] and probably does not encode a critical protein, although it may represent a non-essential facultative protein. If this is the case, it raises the interesting question of why the $\theta 1$ gene has been so well conserved throughout mammalian evolution.



The normal human α-globin complex. The heavy black rectangle below depicts the extent of the South-East Asian deletion.

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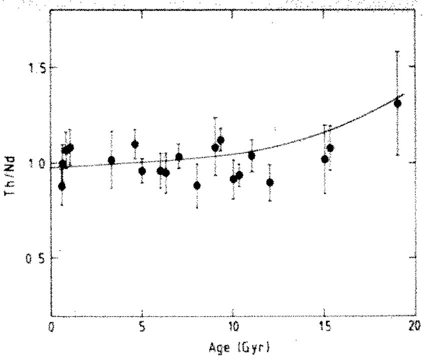
Th/Nd abundance ratio in the surfaces of G-dwarfs

SIR—In a stimulating recent article, Butcher[1] makes wide-ranging conclusions about the age of the Galaxy from his observations of the essential constancy of the Th/Nd abundance ratio in the surfaces of G-dwarfs of varying age. He argues that in the simplest model of chemical evolution for the Galaxy, one in which the nucleosynthesis rate per unit mass of interstellar medium is constant so that stable-element gas concentrations rise linearly in time, the ratio of the concentration of Th to that of a coproduced stable element observed today in a G-dwarf that had formed at time T rises as $(e^{\lambda T}-1)/\lambda T \cong 1 + 0.025T$. Accordingly, in terms of the age of the star (galactic age $-T$) the surface concentrations today decrease with increasing age. His data, reproduced in the figure, do not decline with age, leading to Butcher's powerful conclusions.

I write in admiring caution to argue that, from a purely theoretical point of view, the simplest model expectation is of opposite sign and matches the data well (the curve in the figure). In the astrophysically simplest model, the r-process abundances are primary and indeed grow linearly in time, but the s-process abundances are secondary, being proportional to the initial stellar metallicity, so that they grow quadratically in time. Because Th/Nd observed today measures Th/(Nd$_r$ + Nd$_s$) at time T, the ratio expressed above should, in terms of the time dependence of this simple model, be divided by the additional factor $[(1-s) + (T/T_\odot)s]$, where $s \equiv \mathrm{Nd}_{\odot s}/\mathrm{Nd}_\odot$ is the fraction of solar Nd that has resulted from the s-process and $T_\odot$ is the solar birthdate. Fortunately the neutron-capture cross sections for Nd isotopes have recently been measured[2], enabling the conclusion that $s = 0.52$ (slightly more than half s-process). Using this value and $T_\odot = 15$ Gyr to match the age distribution of stars in the figure results, with no arbitrary parameters, in the curve shown there as the simplest *a priori* expectation for this concentration ratio. This result differs in two important ways from the monotonic decline used in Butcher's version of the figure. First, the ratio observed today is expected to be essentially constant for stars with ages between zero and about 12 Gyr. Second, the ratio is expected to turn upward for stars older than 15 Gyr. Both features seem to me to match the data of the figure better than the monotonic decline used by Butcher. With these assumptions the expectation would therefore be that the observed Th/Nd ratio should not be useful for galactic chronometry, except for its validation of those assumptions. The distribution in the figure is not strongly dependent upon the assumed galactic age, but the upturn in the oldest stars would be still greater for a younger Galaxy. Clearly, a Galaxy as old as 20 Gyr, as estimated from the stellar ages, is not in conflict with this simple expectation, which makes it more in tune with the old ages derived from the discovery two decades ago of the Re/Os chronology[3].

Let it be clear that I in no sense imply that Butcher has erred, for his article shows clearly that he understands and admits this possible loophole in his argument, but that he prefers to believe the implications of his own prior studies[4], suggesting little variation of the s/r ratio in old stars. However, a constant s/r ratio would, in my view, be astrophysically complicated rather than simple. My own prejudice for theoretical simplicity draws some support from observations[5,6] that in the oldest metal-poor stars the r-process has occurred prior to the s-process and has accordingly grown faster initially. Thus the spirit of my remarks is not criticism but rather caution — that the profound conclusion that Butcher has given rests ultimately on understanding a major astrophysical puzzle concerning the relative growth rates of s and r abundances with galactic age and upon the conviction that no systematic misunderstanding compromises the difficult interpretation of spectroscopic line strengths that suggest the absence of differential growth[4].

This caution cannot be easily relaxed by galactic infall. I have presented elsewhere[7] analytic models of the chemical evolution in the face of time-dependent metal-poor infall in which the radioactive concentrations are analytically expressed[7], as are also the secondary metallicities[8]. As long as these models yield monotonic temporal growth of galactic metallicity, they also yield a curve similar to that in the figure.



Th/Nd line-strength ratios versus stellar age[1]. Solid curve: calculation presented in this work when normalized to fit the young stars. This *a priori* expectation is not in conflict with the old Galaxy required by these stellar ages.

This work was supported by the Robert A. Welch Foundation, by NASA, and by Durham University. I thank St. Mary's College, Durham, for a research fellowship that supported my residence there during my sabbatical leave from Rice University.

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BUTCHER REPLIES—As Dr Clayton points out, a judicious choice of s-process abundance evolution over time in the Galaxy can reproduce my data while retaining a large maximum age. In this case, however, one predicts that the ratio of r- to s-process abundances for stable elements will vary in a certain way among stars similar to those in my sample. Variations of the necessary magnitude — a factor of three or more for europium (91% r-process) to barium (84% s-process)[1] — are clearly ruled out by abundance data published elsewhere[2]. This conclusion could only be compromised if the shapes of the abundance curves evolved so that Eu/Ba remained constant while Th/Nd at production changed. I deem the latter unlikely, if not yet completely excluded. My suggestion, therefore, is that undetectable abundance evolution in both radioactive and stable species is evidence for a total timescale much shorter than the relevant decay time, regardless of whether one has an extended production history or not.

The trouble with all these discussions is that the theory of the chemical evolution of the Galaxy by stellar nucleosynthesis has too many unknown parameters, and does not provide much in the way of testable predictions. Therefore, whether a particular development of s- and r-process abundances, with a very large age, is the simplest model must, at this stage, be a matter of taste. Another simple model, for example, which fits available data particularly well except for the stellar-age scale, postulates an initial event with only the most minor ongoing synthesis thereafter. Neither model addresses the curious observation that stars of a given age can vary by factors of five in their heavy-metal-to-hydrogen ratios, while the relative abundances of elements produced by different nuclear processes show undetectable variations. The former suggests mixing of the interstellar gas is not very efficient on short timescales; the latter requires not only efficient mixing, but also a very special production history if synthesis is an ongoing phenomenon.

It seems certain that stars make heavy elements by nuclear reactions in their interior, but how this fact translates into the chemical evolution of the interstellar medium remains vague. My results emphasize that short total timescales and element-production histories not simply tied to the stellar birthrate may well be the preferred possibilities.

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Aluminium and cooking

SIR—Since Savory *et al.*[1] reported far less leaching of aluminium from utensils in the presence of fluoride than we did[2], we have re-examined our experiments and discovered that our aluminium estimates were in error.

We now agree with Savory *et al.* that there is minimal leaching of aluminium in the presence of 1 p.p.m. fluoride, although at 10 p.p.m. fluoride and above, leaching becomes significant. We have also observed a synergistic effect of chloride. We still believe that fluoride-induced leaching of aluminium may in some circumstances be relevant to the possible cumulative toxicity of aluminium.

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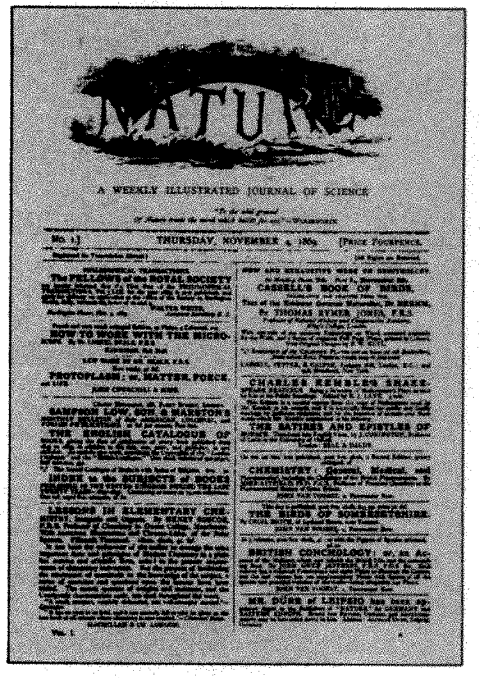

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Genetic machinations

Peter Newmark

Invisible Frontiers: The Race to Synthesize a Human Gene. By Stephen S. Hall. *Atlantic Monthly Press, New York: 1987. Pp.334. $19.95. To be published in Britain next March by Sidgwick & Jackson.*

AT THE end of the summer of 1978, rumours began to spread that one of Britain's top-secret laboratories had been penetrated by foreign agents. This was not the stuff of *Spycatcher*, however, for it was by invitation that four Harvard scientists were spending a month in the Ministry of Defence's Microbiological Research Establishment at Porton while they attempted to clone the human insulin gene.

Two pressures drove them to Porton. The first was that the good folk of Cambridge, Massachusetts, led by their mayor, Alfred Vellucci, and supported by such Harvard scientists as George Wald, were still in the process of holding back our heroes from the frontiers of recombinant DNA research. The result was that no high-containment laboratory had been constructed at Harvard, whereas Porton, with its need to handle highly pathogenic micro-organisms, had such a laboratory available.

The second pressure came from the knowledge that two other groups were also in pursuit of the insulin gene (strictly speaking, it was not the gene itself, but simply the DNA encoding insulin that was being sought). One of the rival groups was centred on the laboratories of Howard Goodman and William Rutter at the University of California, San Francisco. The other was associated with the fledgling biotechnology company, Genentech, whose scientific founder, Herb Boyer, was on the same campus.

As described by Stephen Hall, the four weeks that Wally Gilbert, who had just become involved in a rival company, Biogen, and his three colleagues spent at Porton were pure Gilbert and Sullivan. There were fittings for gas masks, the extreme measures that had to be taken on entering or leaving the high-containment laboratory, and the clash of attitude (and attire) between the regiment of 'gentleman' scientists of the British military establishment and the regimentally casual Harvard men — and women, God forbid! And then contamination of the hard-won human DNA by rat DNA scuppered the purpose of the whole trip.

Worse still, while the Harvard team was struggling in Porton, Genentech announced the production of human insulin from chemically synthesized genes for the hormone's two peptide chains. The use of synthetic genes cleverly avoided the need for stringent containment facilities when it came to cloning and expressing them. Success came from a collaboration between City of Hope scientists, Arthur Riggs and Keiichi Itakura, who had already synthesized a somatostatin gene with Genentech's backing, and the company's own scientists — still without a laboratory of their own — Dennis Kleid and David Goeddel. The result was a brilliant press for Genentech and several million dollars of backing from Eli Lilly, which was the real stimulus to Genentech's rise.

End product — Eli Lilly's advertisement for Humulin, genetically engineered human insulin, the outcome of the events described in Hall's book.

Courtesy Eli Lilly

At the time of Genentech's success, the University of California, San Francisco, was also backed by Eli Lilly. Indeed, one of Goodman's post-doctoral students, Axel Ullrich, was making use of the company's high-containment facility in France. He was there for the same reason that the Harvard team were in Porton and was meeting with as little success. Later, Ullrich and his compatriot Peter Seeburg, who was cloning growth hormone genes, were to break with Goodman and join Genentech.

Stephen Hall is a journalist, and he unravels the various threads in the drama with great skill. His sources are numerous interviews with all the major actors, apart from Howard Goodman, who declined, and with many of the bit players. We are reminded of the days when molecular biologists lost their innocence and bought suits. When venture capitalists struggled to learn the difference between exon and Exxon. When pharmaceutical companies, by and large, concluded that molecular biology had little to offer them. And when public-interest groups and regulators busied themselves saving the world from being ravaged by man-made bugs.

One incident that sums up much of what was worst about the times is recalled, if not resolved, by Hall. Late in 1976, Ullrich was anxious to begin cloning the rat insulin gene. The obvious way to proceed was to make use of plasmid pBR322, which had been designed for cloning in Boyer's laboratory. The problem was that the regulators at the National Institutes of Health were treading very warily in the face of public suspicion and had not cleared the plasmid for use. In mid-January 1977 Boyer received news that the plasmid had been "approved but not certified". Ullrich, in circumstances that remain uncertain, took this as the signal to proceed with cloning.

Success came quickly. But so did the inescapable realization that the plasmid should not have been used before certification. A series of anguished discussions led to the decision to destroy the clones in mid-March. One month later a plasmid that was almost as good as the pBR322 was certified and, with astonishing speed, Ullrich re-cloned the rat insulin gene. The result was announced before its publication at a full-scale press conference which, apart from being a wild success, taught Ullrich and others that postdocs often toil for the glory of their laboratory heads, sometimes when the head has had nothing to do with the work, or has even been in opposition to it.

Hall's book is written in racy style and is bound to meet with distaste in some quarters. It not only has heroes but also villains, of whom Howard Goodman is cast as the archvillain. Indiscretions from scientists who no doubt now regret having been so frank are all gleefully recorded.

As a guide to how scientists really operate, *Invisible Frontiers* is not in the class of *The Double Helix*. It lacks Watson, the scientist–author, his whimsy and his women — the only women in Hall's book are scientists and, for all he says, none of his scientists seem to have partners or indeed any life outside the laboratory. Nonetheless, this is a book that captures a good deal of the spirit of the times and that should enliven many a two-hour plane flight. ☐

Peter Newmark is Deputy Editor of Nature.

Theoretical reality

S. Roy Caplan

Water Movement Through Lipid Bilayers, Pores, and Plasma Membranes: Theory and Reality. Vol. 4, Distinguished Lecture Series of the Society of General Physiologists. By Alan Finkelstein. *Wiley: 1987. Pp. 228. $39.95, £36.65.*

AN eminent reviewer of the time recommended that Guggenheim's treatise *Thermodynamics* should be subtitled *Pride and Prejudice*. Without implying a parallel, this remark all too frequently comes to mind while reading Dr Finkelstein's monograph. The pity is that what might have been an excellent source of information is marred by the author's crotchety preconceptions in assessing the contributions of nonequilibrium thermodynamics to his field.

The work is divided into three parts. Part I ("Theory") accounts for virtually half of the text, encompassing both porous and non-porous ("oil") membranes. The remaining half is apportioned evenly between Parts II and III. Part II is devoted to a detailed treatment of the pores generated in lipid bilayer membranes by antibiotics, while Part III deals with the red cell membrane and with epithelia. The latter half of the book contains the most critical assessment of data on physiological water movement available. It is, however, coloured by the views expressed in Part I.

As Dr Finkelstein points out, there is currently no satisfactory theory to describe solvent flow in pores of molecular dimensions. He avoids mentioning that the concept of pressure in such pores is ill-defined, but rather presents us with explanatory diagrams displaying pressure profiles. These intuitive schemes may certainly provide a mental crutch. For the author, however, they constitute reality, prompting him to castigate nonequilibrium thermodynamics for its ability "to obfuscate the physics underlying osmotic transport through a porous membrane". Thus osmotic flow across a membrane separating solvent and a solution of an impermeant solute, both at the same pressure, is said to be driven simply by a pressure gradient within the pore. If this is the whole story, surely the opposing *infinite* pressure gradient (as drawn) at the pore exit also needs explicit consideration.

The picture is extended to the well-known experiment by Meschia and Setnikar supposedly demonstrating water flow in the wrong direction across a collodion membrane separating moderately concentrated urea and dilute dextran at the same pressure. Osmosis proceeds from urea to dextran, against the chemical potential gradient for water. The notion that solute flux may be coupled to, and hence may drag, solvent flux "completely distorts reality" in Dr Finkelstein's eyes. Not so, he says, water drags urea because the pressure gradient within the pore forces the solution towards the dextran side. How it forces it out of the pore against the large opposing pressure gradient is left for the hapless reader to figure out.

In case of any lingering doubts, Dr Finkelstein asks us to consider a huge sewer pipe with the urea solution at one end, under slight pressure, and pure water at the other. This is claimed to be "exactly comparable" to what occurred in the Meschia and Setnikar experiment. But the comparison is preposterous. The sewer pipe is not of molecular dimensions, and no opposing pressure gradient exists anywhere. If Dr Finkelstein had read the papers of Kedem and Katchalsky he quotes more closely, he might have noticed that the force driving volume (not solvent) flow in their formulation is the overall pressure difference less the osmotic pressure difference of impermeant solute. This correctly describes the volume flow in both the collodion membrane and the sewer pipe. Is this obfuscation?

Nevertheless, I would urge all interested in this problem to buy Dr Finkelstein's book. Nothing better is around. □

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Fighting fit

John R. Krebs

Animal Conflict. By Felicity Huntingford and Angela Turner. *Chapman & Hall: 1987. Pp.448. Hbk £30, $59.95; pbk £14.95, $27.50.*

THIS useful introductory book covers much the same ground as Roger N. Johnson's admirable *Aggression in Man and Animals* (W.B. Saunders, 1972), although it is of course much more up to date. It gives a balanced review of aggression and related behaviours in the animal kingdom and considers various theories about both the causes of aggression and its functional significance. The final chapter moves with due caution from animal studies to human aggressive behaviour. Probably the main messages from the book are: (a) in wild animals aggressive behaviour is not a pathology but part of normal adaptive behaviour; (b) different kinds of aggression (predatory, defensive, competitive) may serve different functions and have different causes; and (c) simple general theories on causes of aggression, popular in the past, are likely to be wrong.

In the 1960s, perhaps not unrelated to the peace movement and rise of flower power, there was a fashion for global theories of human aggression: it was argued that aggression is caused by frustration (never thwart your kids and they will grow up as peace-loving citizens), by pain (do away with corporal punishment and kids will stop being aggressive), by copying role models (cut out violent television movies) and so on. We now know that all these ideas are too simple as overall theories of aggression. A widely publicized ethological contribution to 1960s theorizing was the one so forcefully and eloquently expressed by Konrad Lorenz in his classic book *Das sogennante Böse* (Borotha-Schoeler, 1963), tamely translated into English as *On Aggression* (Methuen, 1966). According to the Lorenzian view, aggression in animals and man is the expression of an inbuilt drive that wells up inside and has to find release in one form or another. The longer it is since you had a meal, the hungrier you feel, and, according to Lorenz, aggressive feelings build up in the same way since your last fight. Lorenz's view, based i part on the correct notion that aggression like feeding, is normally an adaptive and essential component of an animal's daily life, is still very influential. Only a couple of months ago I read a newspaper article on football crowd violence which argued that to suppress violence on the terraces would be merely to cause it to erupt elsewhere — the classic Lorenzian theory.

Sidestepping the difficult issue of how and if at all football hooligan aggression is related causally or functionally to other kinds of fighting, one thing is clear: ethological research has shown during the past 20 years that the Lorenzian drive model of aggression in animals is wrong. As Huntingford and Turner make clear, aggression in animals is a form of competition (usually with members of the same species) for limited resources of food, mates or shelter. The evidence shows that the extent to which animals compete by fighting for resources, as opposed to competing by non-aggressive means such as simple exploitation, depends on ecological circumstances. Both within and between species, the extent of aggressive competition depends on the distribution of resources: when they are localized, animals tend to defend them and fight; when resources are scattered or occur unpredictably in time and space, animals tend not to compete by aggression. If anyone wants to use ethological arguments as a starting point for building a new general theory of aggression, this view of aggression as a flexible strategy, rather than the notion of an inevitable internal build-up, should be the starting point. □

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Try to see things differently

Stuart Sutherland

Vision, Brain, and Cooperative Computation. Edited by Michael A. Arbib and Allen R. Hanson. *MIT Press: 1987. Pp. 730. $65, £58.50.*

ONLY 20 years ago it was possible to keep up with all the research published on vision, with the possible exception of colour vision — always an arcane speciality. Today there is so much work done in each subdivision of the field that it is impossible to keep abreast of it all: indeed it has become difficult to cope adequately even with one of the many subdivisions such as motion perception, stereopsis, eye movements, the extraction of lines and edges from the retinal image, the use of motion to form a three-dimensional description of an object, the ability to construct a representation of a scene from glimpses obtained through successive fixations, and the recognition of patterns and objects.

Vision, Brain, and Cooperative Computation deals with all these topics and many more, and as the title implies it does so from the point of view of psychology, neurophysiology and artificial intelligence. The book is not for the faint hearted, for it would take a polyopticist to understand in depth its highly technical chapters covering such a wide range of phenomena and ways of thought. Nor do the editors, Michael Arbib and Allen Hanson, do much to help, for the graphs are often poorly labelled and captioned, and much of the writing is slovenly. Take for example the sentence "The level of formulation is in terms of symbolic constraints and algorithms for solving them": surely one does not solve constraints though one may use constraints to solve problems. Also, the book is so full of abbreviations that parts of it are more akin to Stock Exchange listings than to prose. It is sometimes difficult to separate what is new from the frequently occurring but magisterial statements of the banal, such as "I suggest that the cortex has adopted representational and computational strategies that make the computation of invariants efficient", a view with which few are likely to disagree.

Nevertheless — and despite the fact that the conference on which the book is based took place four years ago — most workers on vision will find scattered through its pages nuggets of information with which they are unfamiliar. Sparks and Jay argue that in the superior colliculus, which is involved in the control of eye movements, cells code for the target position of the eye in its orbit, not simply for the retinal distance and direction from the fixation point of the target to be fixated. If the target is briefly illuminated and an eye movement occurs before the fixation movement to the target, account is taken of the new position of the eye.

A more dramatic and better known neurophysiological result is Spinelli's finding that cats exposed only to vertical lines on one eye and only to horizontal on the other develop central cells that are maximally stimulated by a vertical line on the former eye and a horizontal on the latter. This result is important because it demonstrates conclusively that learning can occur at this level of the visual system — the combination of lines is one that cannot occur in nature.

On the psychological side, Weisstein and Wong review some elegant experiments showing that judgements of orientation are much better for lines embedded in a part of the scene that is taken as figure than for lines on what is taken as background. Moreover, while sharp lines are more readily detected when inside a figure, blurred lines are more readily detected when in the background. In both cases the lines were fixated, so these results are not due to differences in retinal position. Under most circumstances fine detail within a figure is important to us, whereas a general impression of the background will suffice. It is nevertheless remarkable that the visual system has evolved to meet these task requirements in so subtle a way.

Readers' hopes will be aroused by a sentence beginning "In an informal study of the motor skills involved in drinking beer . . .", but alas the findings have no practical application, particularly as they concern only the replacement of the beer mug on the table, not the action of picking it up. In replacing it, there is a fast movement to a point above the table followed by a slower movement that lowers it gently on to the table. The distance above the table at which the fast movement ends increases with increased alcoholic intake, thus allowing a greater margin of error — again a nice example of the way in which the human brain adapts, though in this case the adaptation is presumably due to learning not evolution.

The chapters on artificial intelligence are so opaque that it is hard to know what to make of them. One presents a network for storing the representations of events in their temporal order, but it gives no consideration to the factors that influence people's memory of temporal order. Another develops a suggestion of Alan Cowey's that the reason why different properties of the visual image are coded in different central cells is that far more cells would be needed if there were different cells coding for each possible combination of properties. Rightly or wrongly I felt that many of these contributions were merely restating a problem rather than attempting to solve it.

Like most books of collected papers, this one fails to make a satisfying meal. But it does provide quite a few tid-bits, some of which are more easy to digest than others. ☐

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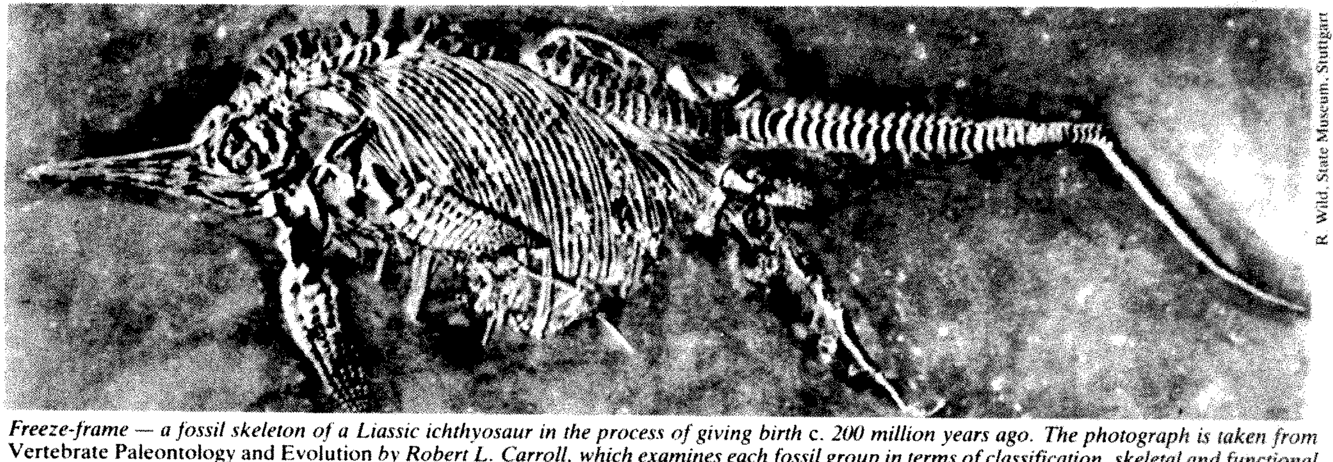

R. Wild, State Museum, Stuttgart

Freeze-frame — a fossil skeleton of a Liassic ichthyosaur in the process of giving birth c. *200 million years ago. The photograph is taken from* Vertebrate Paleontology and Evolution *by Robert L. Carroll, which examines each fossil group in terms of classification, skeletal and functional anatomy, distribution and ecology. The book has over 1,700 illustrations, is just published by W. H. Freeman and costs $52.95, £47.50.*

Rumblings from Hawaii

Floyd W. McCoy

The Volcano Letter. Reprint edition edited by Richard S. Fiske, Tom Simkin and Elizabeth A. Nielsen. *National Museum of Natural History/Smithsonian Institution Press*: 1987. 3kg. $30.*

SEVENTY-FIVE years ago, Thomas A. Jagger settled in Hawaii to study volcanoes; he had become interested in volcanic hazards after seeing the devastation of St Pierre, Martinique, following the 1902 eruption. The Hawaii Volcano Observatory (HVO) was thus in business. To raise funds, Jagger circulated a weekly newsletter to donors — *The Volcano Letter*. The newsletter described tilt measurements and eruptions on Kilauea and Mauna Loa, seismic activity throughout Hawaii and how to make a seismograph; it discussed all aspects of volcanism; it chronicled Jagger's travels; and it catalogued world-wide seismic and volcanic activity. *The Volcano Letter* was thus a curious mix of comment, data and observations.

The Volcano Letter lasted 31 years. It was published in various formats depending upon changing financial circumstances and publishers. Upon Jagger's retirement in 1940, Ruy Finch became editor. With Finch came more scholarly articles, less chatty and with references (an equation printed in 1941, in issue No. 473, signals writing directed to a new audience). Gordon Macdonald followed as editor until 1955 when the last issue was printed. *The Volcano Letter* was left to reside in libraries, increasingly forgotten except by those devoted to Hawaii and its volcanoes.

No more. The entire publication, 530 issues, has been reprinted in one bound volume, fully indexed, to coincide with the seventy-fifth anniversary of HVO. What delightful reading it is — here is a history, a genealogy of research on Hawaiian volcanism. Read Jagger's "Native Superstition" (No. 431) after the bombing of the 1935 Mauna Loa flow heading towards Hilo: the Hawaiians predicted that in "anger the goddess [Pele] would immediately vent her wrath upon [Hilo] and cause widespread destruction. [But] an aged native . . . stopped the flow by direct supplication to the goddess. This gentleman claims direct descent from the goddess herself . . .".

Here are Jagger's predictions of doom for Hilo by either lava flow or tsunami, or both, that culminated in the 1946 tsunami disaster. He pleaded for a Pacific warning system, and for lava barriers above Hilo, insisting that "The shadow of the lava menace is just as removable as the shadow of malaria, tuberculosis or yellow fever, the noxious mosquitoes . . ." (No. 443).

The Volcano Letter also contained narratives of eruptions. For instance, the 1880–1881 Mauna Loa flow (No. 520): "We cooked every meal on the lava . . . Occasionally our frying pan floated down with the lava . . . the flow crossed [a] stream, forming a natural . . . bath, from cold to scalding hot". Or this account from Kilauea in 1936 (No. 441): "A thousand people at the active crater . . . are standing . . . on the edge of a vast circular pit . . . motionless . . . a concourse of fire worshippers. All are looking down, over a ring of precipice 800 feet high" — some things never change.

Equally fascinating are Jagger's comments on continental drift; or on the need to explore the seafloor: "Why go to remote lands when the greatest discoveries in the history of science and invention are within . . . a sampan sail of thirty knots from Hawaii" (this comment was made in 1925, in No. 49).

Wonderful stuff. Here are Jagger, Finch, Powers, Stearns, Wentworth, Stone, Palmer, Macdonald and others talking about their science as much as they are reporting it. But primarily here is a chronicle of one man, Jagger, and his times — a scientist and his interests, an observatory and its development. At $30, a copy of the book should be in all libraries serving geologists and geophysicists concerned with volcanism or the history of science. ☐

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* Orders to E. Nielsen, NHB Mail Stop 119, Smithsonian Institution, Washington, DC 20560, USA.

Sensitive science

Martin Smith

Biosensors: Fundamentals and Applications. Edited by Anthony P. Turner, Isao Karube and George S. Wilson. *Oxford University Press: 1987. Pp.770. £60, $110.*

BIOSENSING is a new field of science which pulls together an enormous range of disciplines, and which is likely to have a considerable effect on clinical testing, environmental monitoring, industrial process control and battlefield early-warning systems. The breadth of the subject and the diversity of the basic science training of existing and prospective practitioners mean that there is the danger of the wheel being re-invented and that constraints on the performance of actual biosensing devices are not appreciated. In addition there is the need to move outside the laboratory and develop sensors that can be manufactured and can live up to the rigours of the proposed operating environment. This is the backdrop to the scene into which *Biosensors: Fundamentals and Applications* makes its entry.

The aim of the editors of this international multi-author text is "to provide the first advanced and comprehensive treatise on the subject of biosensors". In seeking to achieve this aim, the range of subjects covered includes all those that can even loosely be described as biosensing, and there are also chapters on related technology areas such as DNA probes and genetic engineering. All of the contributions are by respected specialists and are well written, and in each area both the fundamental science and the most recent attempts at practical realization are presented. No attempt is made to make judgements as to which areas of technology are most likely to succeed, which is a realistic approach in view of the embryonic nature of much of the work. However, the level of coverage is such that the skilled reader is well placed to reach his own conclusions.

The book leads naturally from a discussion of principles, through detailed chapters on the basic methods of biosensing (bioelectrochemical, acoustic, calorimetric and photometric), to accounts of instrumentation aspects and commercialization. As would be expected, over half the text is devoted to the many different electrical and electrochemical methods which have been investigated. In many cases the approach taken in expounding each technique is not new, but even the expert reader will find it helpful to have discussion of them brought together in a single tome. Although the coverage is academic and comprehensive, if read selectively the text is nonetheless suitable as a general introduction for the non-specialist reader.

No one can tell where biosensor technology will be in ten years time. The editors of this book make no predictions and neither would I. But it is highly likely that this work will still be a valuable foundation text whichever way the technology progresses. ☐

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- A Discussion on Biosensors, edited by M. Akhtar, C. R. Lowe and I. J. Higgins, is presented in *Phil. Trans. R. Soc.* **B316**, 1–181 (1987). This issue is also published as a clothbound book entitled *Biosensors*, available to subscribers to *Philosophical Transactions* at £19.80 (UK), £21 (elsewhere), and generally available at £33 (UK), £35 (elsewhere) from the Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, UK.

NATURE VOL. 329 1 OCTOBER 1987

Vostok ice core: a continuous isotope temperature record over the last climatic cycle (160,000 years)

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A continuous deuterium profile along the 160,000-year Vostok ice core (Antarctica) is interpreted in terms of atmospheric temperature changes. This climatic record is the awaited terrestrial complement of the deep-sea records supporting the existence of a relation between the Pleistocene climate and orbital forcing.

On the timescale of the successive glacial-interglacial episodes that have characterized the Earth's climate for about the past million years, quantitative reconstruction of climatic parameters essentially relies on oceanic records, most notably isotopic and faunal studies of deep-sea core sediments. These records provide time series of the continental ice volume and of sea surface temperatures[1-3] and have convincingly demonstrated that there is a link between the Pleistocene ice ages and the variations in the elements of the Earth's orbit. By contrast, the climatic conditions that prevailed over continental areas are far less well documented and rarely on a quantitative basis. However, such continental time series are essential to an understanding of how the atmosphere-ocean-cryosphere climatic system is operating on this timescale.

In this context, the 2,083-m ice core[4] recovered by the Soviet Antarctic Expeditions at Vostok (East Antarctica) is of fundamental importance because it fully covers the last glacial-interglacial cycle, back to the ice age that preceded the last interglacial (160 kyr BP) and has been essentially undisturbed by flow conditions. It allows access to many climatic and climate-related parameters. These potentials are illustrated from the ^{18}O data we have recently published[4], from ^{10}Be measurements[5,6], from the aerosol concentrations[7] and, in a companion paper, from the CO_2 profile[8].

Our first isotope dataset was largely discontinuous over the past 100 kyr (only 7% of the core was analysed), and continuous below that. Sampling of the ice was completed later in the field and complementary isotopic measurements were made. We present here continuous data for deuterium throughout the core. We shall focus first on the interpretation of this profile in terms of temperature and then examine this continuous record in the frequency and time domains. We shall compare the ice record with the oceanic record, with a special emphasis on the Last Interglacial Period. For the first time an ice core reveals the large 100-kyr glacial-interglacial cycle and concentration of variance near the Earth's orbit tilt and precession frequencies.

Continuous deuterium profile

The samples were analysed both for their ^{18}O and deuterium contents. Although uncommon in the deep-ice-core literature[9-12], the present interpretation is based on the deuterium record (denoted δD and expressed in per mil with respect to standard mean ocean water (SMOW)). This is justified because δD is, as discussed later, a slightly better indicator of temperature change than δ^{18}O.

The basic characteristics of the Vostok site are given in ref. 4. The new samples were taken along 1-m ice increments (instead of 2 m) and were processed as described in ref. 4. The data are now available on an almost continuous basis on the 2,083-m core below 138 m and a complementary dataset was obtained on a new adjacent 279-m core. The total ice recovery is about 85% with only one long (8 m) missing series. Data obtained from the two cores (with an accuracy of 0.3‰ w.r.t. SMOW) are in excellent agreement over their common part (within 1‰ for the average values) and the two series of results were combined to obtain the deuterium profile shown in Fig. 1 as a function of depth.

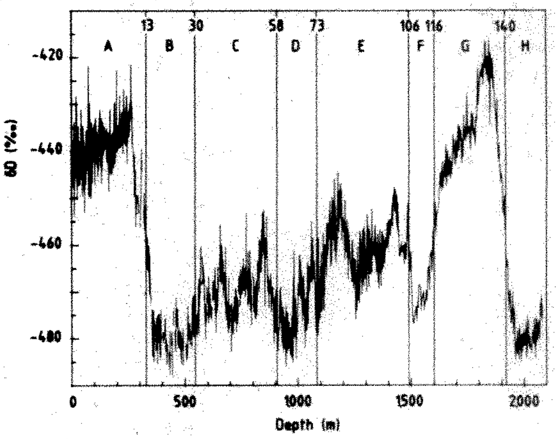

Fig. 1 Deuterium content (in ‰ w.r.t. SMOW) against depth in the Vostok ice core with successive stages as defined in ref. 4 and the ages corresponding to the limits between these stages.

There are obvious similarities between the present δD and the previous δ^{18}O Vostok record with successive warm and cold stages indicated in Fig. 1 and designated by letters from A to H (ref. 4). In the upper part of the figure are also reported the ages corresponding to the limits between stages, following the Lorius *et al.*[4] chronology. This chronology, established with a two-dimensional ice-flow model and taking into account the change with time of snow accumulation, is discussed in further detail in the last section.

Isotope temperature record

Preliminary estimates of atmospheric temperature changes in the Vostok area were given from the available δ^{18}O data[4]. For completing this approach from the continuous δD record, it is useful to examine the validity of this reconstruction from central East Antarctic deep ice cores[4,11], which relies on both experimental and theoretical arguments.

The deuterium content distribution in recent snow is well documented over East Antarctica, in particular along the

Dumont d'Urville-Dome C axis[13]. Over a large range of temperature (−20 to −55 °C) there is a linear relation between the annual averages of Θ_s, the surface temperature, and of the snow deuterium content with a δD-Θ_s slope of 6‰ per °C, which approximates very well all the data along this axis. As already noted by Lorius[14], there are some regional features in the isotope distribution over Antarctica, but this value is in the range of that which we recently determined (unpublished results) along the Vostok-Mirny axis (slope of ~7‰ per °C). These slopes are also in the range of that encountered in other parts of Antarctica and in Greenland with an average value of 0.7 ± 0.3‰ per °C in $\delta^{18}O$ (ref. 15) (that is ~5.6‰ per °C in δD).

From a simple one-dimensional isotope model, Jouzel and Merlivat[16] have demonstrated that there is agreement between these observations and theoretical predictions, provided one takes as the formation temperature (Θ_1) that prevailing just above the inversion layer[17]. There is, on an annual basis, a linear relation between Θ_1 and the surface temperature, Θ_s:$d\Theta_1/d\Theta_s = 0.67$. This model accounts for the kinetic fractionation that occurs because snow formation takes place in an environment supersaturated over ice. Indeed, the classical Rayleigh model assuming isotopic equilibrium leads to temperature-isotope gradients that are too high over central East Antarctica. These gradients are reduced when the kinetic effect is considered, down to values around 6‰ per °C for δD (with respect to Θ_s), in good agreement with observations.

This slope reduction is relatively important for $\delta^{18}O$ (up to 50%) and relatively marginal for δD ≤20%). Obviously, a change with time in the relative strength of this kinetic effect, possibly associated with changes in saturation conditions, could partly obscure the relation between the isotopic content of polar precipitation and its temperature of formation. Changes in the kinetic effect are isotopic noise in the sense that they are not directly temperature-related. Their relative influence is weaker for δD than for $\delta^{18}O$. The same is true for the kinetic effect associated with sea-surface evaporation, which is dependent on relative humidity[18,19]. It influences the isotopic content of the vapour leaving the oceanic surface and eventually that of the resulting precipitation, but again relatively less for δD than for $\delta^{18}O$. This is why, as far as both sets of data are available, we prefer to use the δD record for temperature reconstruction.

The distribution of water isotopes in modern precipitation was also recently investigated by two different groups[20,21] using General Circulation Models of the atmosphere. A simulation of the full annual cycle was performed with the GISS (Goddard Institute for Space Studies) model[21], and the results can be directly compared with the Antarctic data. The deuterium minimum is well represented over central East Antarctica. The predicted deuterium-surface-temperature gradient is 6.5‰ per °C, very close to the observed one.

Based on these considerations, the past surface-temperature estimate is obtained with a gradient of 6‰ per °C after correcting the Vostok deuterium profile for the change in δD of the oceanic water (the correction is based on the stacked $\delta^{18}O$ record of ref. 3). Figure 2*a* shows the isotope surface-temperature record. Superimposed on the detailed record obtained after equally spaced resampling every 100 yr is a smoothed curve in which much of the very high frequency oscillations are filtered out. This surface-temperature record is given as a difference with respect to the present value ($\Theta_s = -55.5$ °C). Note that the corresponding atmospheric temperature changes would be approximately two-thirds lower above the inversion layer.

We shall now examine the validity of applying the present deuterium-temperature gradient, well documented and theoretically explained for modern snow on a spatial scale, to an interpretation of the isotopic content of past precipitation, that is, on a temporal basis. This approach requires several conditions to be fulfilled, in particular, that all along the record (1) the influences of factors other than the temperature of formation of precipitation, that is, conditions of temperature and relative humidity in the moisture source regions and shifts of these regions, further dynamical and microphysical history of the air masses, are negligible or at least cancel statistically; (2) the relation between inversion and surface temperatures remains unchanged; (3) there is no additional isotopic noise introduced through variations such as the summer : winter ratio in precipitation amount, which could itself be time-dependent[15].

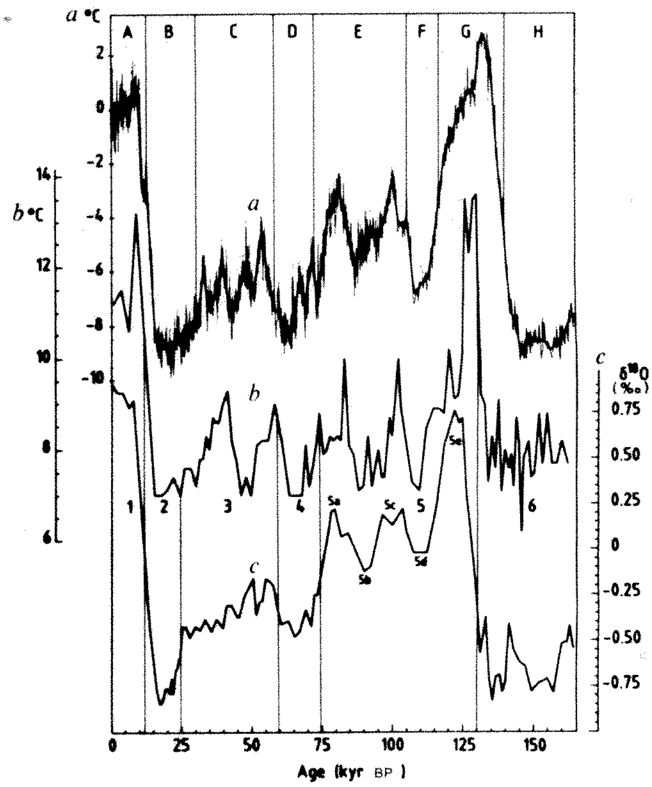

Fig. 2 *a*, Variation with time of the Vostok isotope temperature record in °C as a difference from the modern surface-temperature value ($\Theta_s = -55.5$ °C). The thin line corresponds to equally spaced values every 100 yr, the thick line to a smoothed curve. Vostok isotopic stages are as in Fig. 1. *b*, Variation with time of estimated summer sea-surface temperature at the subpolar Indian ocean site RC11-120 (from ref. 3). *c*, Variation with time of the stacked $\delta^{18}O$ record of ref. 3 (normalized series) with limits between successive marine stages as defined by Martinson *et al.*[3], plus an indication of substages 5a to 5e.

There are no strong climatological arguments that the above requirements were fulfilled for conditions as different from each other as those prevailing during glacial and interglacial periods. Noting that modern Antarctic and Greenland precipitations are also formed under different climatic conditions, the overall variability of the present surface gradients (~40% from ref. 15, including both spatial and temporal gradients) may be considered an upper limit of the relative error associated with our temperature-change estimates. Indeed, a recent interpretation[22] of crystal size variations with depth in terms of surface-temperature change indicates that this uncertainty is probably much smaller. At Vostok the change associated with the last deglaciation is estimated as 11 °C from the crystal size, which compares well with 9 °C in our present interpretation (that is, a relative difference of 20%). Our figure is also consistent with a recent simulation of the Last Glacial Maximum by Broccoli and Manabe[23]. Additionally the ^{10}Be profile allowed an estimate of past precipitation[5]. The results are in good agreement with those obtained assuming that precipitation is controlled by saturated vapour pressure[4]; this would not be so if our temperature interpretation of the isotope record was not correct.

Taken together the arguments developed in this section become convincing that much of the isotopic signal has its origin

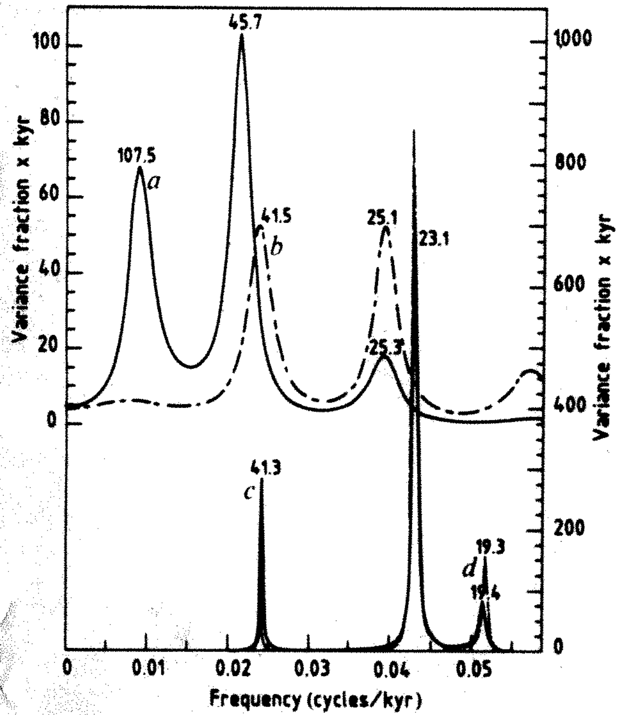

Fig. 3 Variance spectra of the Vostok isotope temperature (*a*, *b*) and of mid-July insolation at 65° N for the past 160 kyr (*c*, *d*). The normalized variance density is plotted against frequency in cycles per kyr on linear scales. The left scale is for curves *a* and *b* and the right scale for curves *c* and *d*. Continuous lines refer to the original series (*a*, *c*) and broken lines to these series after prewhitening by a first-order difference filter (*b*, *d*). These four spectra were obtained by the maximum entropy method applying the Barrodale and Ericksson[33] algorithm with an autoregressive order of 40%.

in the variation of local surface temperature. On the other hand, deviations of up to 20% in the estimate of the temperature change cannot be excluded. Finally, to get the climatically induced temperature change, corrections have to be applied for the changes of elevation either due to the motion of the ice from its origin or to general changes in the thickness of the ice sheet[24]. We now have new isotopic results from snow surface samples both at Vostok and Ridge B, that is, over the region from which the Vostok ice probably originated over the last climatic cycle[4]. The two average deuterium values for about the last century fall within a few per mil (−438‰ at Vostok and −435‰ at Ridge B). This confirms that there is no need for a correction linked with the origin of the ice. The available data on ice cap thickness (D. Raynaud, personal communication) show that there are variations in the total gas content of the ice, a parameter directly related to the elevation of the ice cap, which are roughly in phase with the deuterium changes. Taking this effect into account would amplify the amplitude of our estimated temperature variations but not the shape of the profile. This aspect is not taken into consideration in the present interpretation but will need to be kept in mind in a final assessment of the data.

Spectral analysis of temperature record

The Vostok ice offers a unique opportunity to examine from a continental record the link existing between a time series for atmospheric temperature and the astronomical forcing that drives the amount of insolation received at any latitude and time. Among the orbital parameters, the obliquity of the Earth's axis (period of ~41 kyr) and the precession of the equinoxes (periods at 23 and 19 kyr) are the most important as they redistribute the available energy between latitudes and months[25-28]. For example, there is about a 20% difference in the mid-July insolation received at 65 °N (a key latitude in the classical theory of the ice ages) between a time of high or low seasonal contrast[25]. On the other hand, the total insolation received by the planet, only influenced by the Earth's eccentricity (periods at 100 and 400 kyr) varies by only about 0.3% over the past 160 kyr. The ~100-kyr oscillation that dominates the climate record over the past million years is generally assumed to result from a nonlinear response of the ice sheets to orbital forcing rather than directly from eccentricity changes[28-31].

Here we focus the spectral analysis on the obliquity and precessional frequency bands. The higher frequency spectra, which also contain information about the behaviour of the climatic system[30,31], will be examined elsewhere. For this analysis we followed the strategy described by Berger and Pestiaux[27], who suggested the use of a combination of different methods, each with its own advantages and drawbacks.

The commonly used Blackman-Tukey[32] technique has well-defined statistical properties and is reliable for spectral-amplitude information but has poor frequency resolution. It is not very appropriate in our case of a record that is short with respect to the periods of interest. Even using a large number of lags (50%), there is no clear indication of spectral peaks.

We improved the frequency resolution using the Maximum Entropy (ME) method by applying a least-squares algorithm[33] appropriate for short records. Figure 3 (curve *a*) shows the Vostok spectrum. The spectrum of mid-July insolation at 65° N for the past 160 kyr (curve *c*) illustrates the quality of this method, which clearly separates the 23-kyr and 19-kyr precessional peaks. To depict the higher frequency part of the record more clearly, the Vostok series was prewhitened with a first-order difference filter, thus removing variance at low frequency (curve *b*). Curve *d* shows the corresponding spectrum for the 65° N insolation.

These spectra were calculated with an autoregressive order of 40%. We checked that the position of the peaks is fairly stable (within ~±3%) over a large range of values of this parameter (from 35 to 50%). The unprewhitened Vostok spectrum (Fig. 3, curve *a*) shows well-defined peaks at 107.5, 45.7 and 25.3 kyr. A much larger part of the variance is concentrated in the ~40-kyr peak than in the 20-kyr peak. After filtering of the series (curve *b*) the 45.6-kyr peak is shifted towards higher frequencies (41.5 kyr), probably because its position was influenced by the strong 100-kyr component. In contrast, the position of the 41-kyr peak for 65° N insolation is not affected by the prewhitening (curve *d*) because there is no such strong 100-kyr component.

The Vostok peaks at ~40 and ~20 kyr are on the lower frequency side of the 41-kyr obliquity component and of the 23-kyr precessional main component but the differences are well within the dating accuracy, which is in the range 5–10% (ref. 4). Note here that consequence of claiming that δD is a better temperature indicator than is $\delta^{18}O$ should be to correct the timescale. The influence of using δD rather than $\delta^{18}O$ in the age model of ref. 1 never exceeds 2%. This is well below the dating uncertainty as the influence of ice thickness probably changes.

Within these limits, our spectral analysis demonstrates convincingly that, apart from the ~100 kyr signal, the Vostok temperature record is dominated by a strong component near 40 kyr previously seen from visual inspection of the record[4]. It also suggests that it is influenced to a lesser degree by a component slightly above 20 kyr. The two frequency bands may be associated with the obliquity and precession cycles respectively. This result is quite remarkable if we recall that the Vostok dating is based only on a glaciological model[4]. When deep-sea core profiles of $\delta^{18}O$ are analysed without any assumption of a relation between an insolation curve and the isotopic record, they generally do not show better defined peaks in the obliquity and precessional bands. For example, Berger and Pestiaux[27], from the study of 10 cores dated in this way, showed that the variability in the position of the 41-kyr and 23-kyr peaks is

⩾5 kyr. At the least, the Vostok atmospheric temperature record appears to be the awaited terrestrial complement of the deep-sea cores to support the role of astronomical forcing in determining the last Pleistocene climate.

Comparison with marine climatic record

Figure 2 (curves *c* and *b*) shows time series of the estimated continental ice volume change and of a Southern Hemisphere sea-surface temperature record for the past 160 kyr. They represent respectively the stacked SPECMAP $\delta^{18}O$ record of Martinson *et al.*[3], with the 'standard' stratigraphy for deep-sea sediments and the estimated summer sea-surface temperature from the very well documented[1,3] and geographically relevant subpolar Indian Ocean core RC11-120. For these two series the chronology established by Martinson *et al.*[3] stems explicitly from orbital tuning. We discuss here the salient characteristics of the Vostok record and its comparison with these two marine climatic series, going back in time from the Holocene to the last Interglacial.

The warmest part of the Holocene occurred just at the end of the last deglaciation, which is also seen in RC11-120 (temperature maximum occurring at ~9,000 kyr BP) and was previously observed in the Dome C core[11]. At Vostok the surface-temperature shift between the Last Glacial Maximum (LGM) and average Holocene conditions is about 9 °C. This transition is a two-step process with two warming intervals interrupted by a slightly colder one lasting ~1 kyr (from ~12 to 11 kyr BP in our chronology). The temperature amplitude of this cold episode is ~2 °C. There is some missing ice in this part of the record, but there is still no doubt that a cold oscillation exists in this Vostok transition as in Dome C[11] and possibly Byrd[9]. The relationship between this Antarctic event and the well-documented European Younger Dryas (see ref. 34 for an extensive review) well recorded in the Greenland deep ice cores[12] is examined in Jouzel *et al.*[35]. According to the best dating so far there is no significant lead or lag between the three climatic curves of Fig. 2 at the beginning of this transition from the LGM towards the Holocene, which takes place in the three records at ~15 kyr BP.

We had interpreted[4] stages B, D and F (which correspond to marine stages 2, 4 and 5d) from $\delta^{18}O$ as being very similar. Figure 2 (curve *a*) shows a decreasing trend of the temperature minima from F to B, with stage F being ~2 °C warmer than the full glacial conditions (stage B). This difference in our interpretation of stage F partly relates to the fact that the deuterium excess ($d = \delta D - 8\delta^{18}O$), largely depending upon the above-mentioned kinetic effects, is then particularly high compared with that recorded during the other cold stages (the continuous deuterium-excess profile will be discussed elsewhere). In fact, conditions equivalent to the LGM were encountered only at the end of the penultimate glacial (stage H).

The two C and E interstadials are now thoroughly documented, demonstrating in each case a well-defined internal structure.

During stage C there are two double-peak maxima separated by a period of colder conditions (but about 1.5 °C above the LGM) at ~42 kyr BP. The warmest conditions prevailed at the beginning of this interstadial (~53 kyr BP) with temperature up to 4 °C above full glacial conditions. There is a reasonable correspondence between these Vostok stage C temperature maxima and the corresponding parts of the oceanic records (marine stage 3). There is in the detailed curve nothing comparable with what is recorded in the Dye 3 Greenland ice core[12], which shows at the timescale of a few decades abrupt and vigorous isotopic changes with an amplitude almost equivalent to that accompanying the Pleistocene-Holocene shift. The lack of such large oscillations in our core is far from proven, given the relatively coarse sampling we used.

Vostok interstadial E is characterized by two well-marked temperature maxima (~81 and 100 kyr BP) and some minor additional oscillations. For both events, the temperature culminates at a level ~6 °C above the LGM temperature. There is quite clear correspondence between marine stages 5a and 5c, seen both in the $\delta^{18}O$ and temperature oceanic record, and these two relatively warm periods. Correspondingly, stage 5b agrees well with a period ~2 °C colder recorded in the Vostok ice from ~95 to 85 kyr BP.

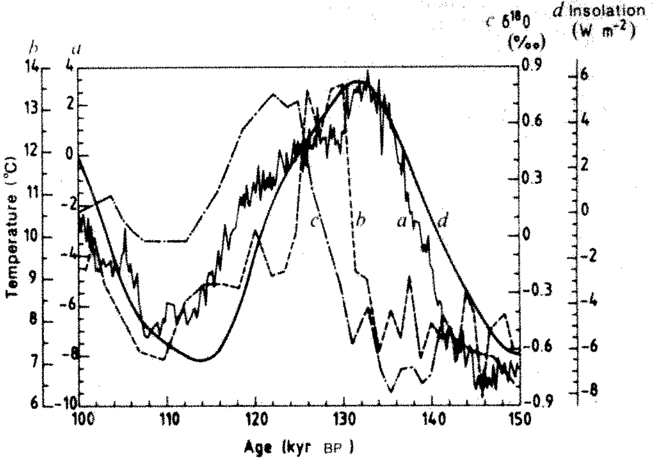

Fig. 4 Variation with time of the three climatic series of Fig. 2 for the period between 100 and 150 kyr BP. *a*, Vostok isotope temperature (thin continuous line); *b*, RC11-120 summer sea-surface temperature from ref. 3 (broken line); *c*, stacked $\delta^{18}O$ record from ref. 3 (dot-dashed line); *d*, annual insolation at 78° S (thick line). The four curves were scaled, for the sake of comparison, to have about the same amplitude.

We note here the remarkable correlation ($r^2 = 0.87$) between the marine $\delta^{18}O$ and the Vostok record down to ~110 kyr BP. As the marine $\delta^{18}O$ record is thought to represent essentially continental ice-volume changes, that is, a parameter of global significance, this high correlation suggests that the Vostok temperature record is, at least qualitatively, of relatively large geographical significance.

Such a general agreement for timescales between the ocean and ice climatic records does not hold for the earlier period of the record (before 110 kyr BP). We shall now concentrate on this period after pointing out that the δD interpretation confirms[4] that the Last Interglacial contains an interval of ~5 kyr warmer (~2 °C) than present conditions. For the sake of this discussion parts of the three palaeorecords from 150 to 100 kyr BP are shown in Fig. 4 (curves *a* to *c*). The major difference is in the duration of the Last Interglacial, namely 11 kyr (127–116 kyr BP) for the $\delta^{18}O$ deep-sea-core stage 5e and about 22 kyr (139–117 kyr BP) for the Vostok stage G. This difference inevitably induces discrepancies between the ice and deep-sea-core chronologies. Some mechanisms like the Antarctic floating-shelves hypothesis[36] could explain such a difference. We shall argue instead that these major climatic changes were nearly synchronous on a global scale. First, we assume[8,37] that apart from astronomical forcing, CO_2 changes that are global have played a climatic role. Second, there is little doubt that the last deglaciation was nearly in phase in both hemispheres[38], as discussed earlier in this section, and it is difficult to justify that a different glacial mechanism with a large phase lag had operated during the penultimate deglaciation. Third, as firmly pointed out by Broecker[38] and J. Imbrie (personal communication), temperature changes over Antarctica must be nearly synchronous with changes in surface temperature conditions around the Antarctic continent.

The deep-sea-core chronology[3] is independently constrained and very well confirmed by dating, in particular, of Barbados high-sea-level terraces, which sets Barbados III level as corresponding to marine stage 5e at 125 kyr BP (refs 39, 40). In disagreement with Barbados terraces chronology, there is an abundant literature[41-43] on Huon Peninsula (New Guinea) sea levels, showing a doubled-peak long interglacial (~25 kyr at mid-peak) with two high sea-level stands at 132 and 120 kyr BP that would fit with the Vostok chronology. Let us follow the CLIMAP project members in their conclusion that these terrace ages must be in error[44]. However, there are apparently reliably dated coral reefs of Haiti that also give a high sea-level stand at 130 kyr BP (ref. 45). Such a possibility is at least not contradicted (the earlier date is 129.9 kyr BP) by recent and more accurate datings using mass spectrometry[46]. These dates leave open the possibility of an earlier interglacial as suggested by the Vostok record. Indeed, as Southern Hemisphere temperature changes are expected to lead sea-level changes by a few kyr (ref. 44) a maximum sea level at 130 kyr BP would be in agreement with the Vostok profile, which shows its temperature maximum at ~133 kyr BP.

In the Vostok chronology underestimation of both the accumulation rate and the thinning function could have led us to overestimate the duration of Vostok stage G (ref. 4). Previously published[5] and new detailed[6] ^{10}Be measurements are consistent with quite similar snow accumulation rates at the peak of the Last Interglacial and during the Holocene. Aerosol input of either continental or marine origin, as estimated from sodium and aluminium measurements, is not very different between the Holocene and the Last Interglacial Maximum[7], indirectly strengthening this argument although changes in atmospheric circulation or in production rates (for ^{10}Be) may certainly not be excluded. The thinning function depends on flow parameters, which may be affected by various factors such as concentration of dust and other impurities and crystal size and fabrics [47]. Preliminary experiments suggest that the Vostok interglacial ice may be only slightly more viscous than glacial ice[48]; a corresponding adjustment leads to only small changes in our chronology.

Over this period, relevant Southern Hemisphere insolation curves show a maximum before 130 kyr BP. For example, this is true for November insolation at 60° S, which partly controls the summer extent of sea-ice around Antarctica[49] and for the annual average of the local insolation shown on Fig. 4 (curve *d*). Indeed, there is a remarkable correlation between this insolation curve and the Vostok temperature over the 150–100 kyr interval. If the Antarctic climate were governed in some way by Southern Hemisphere insolation changes (ref. 50; G. Kukla, personal communication), this would tend to support the Vostok chronology.

Thus we think that there is no convincing argument to modify the Vostok timescale of ref. 4, which is adopted here and in the companion papers[8,37]. This choice does not mean that we deny the role of orbital variations in influencing the Earth's climate; indeed the application of orbital tuning techniques to the Vostok record is being explored in collaboration with J. Imbrie. But we do claim that the Vostok ice core offers a unique source of information which will be most valuable if dated independently of the deep-sea core record.

Conclusions

We now have obtained a continuous deuterium record along the Vostok ice core that we have interpreted in terms of local surface temperature changes over the past 160 kyr. This is dominated by the large ~100 kyr glacial–interglacial signal with a total temperature amplitude of ~11 °C; the Last Glacial Period is very well documented and it is confirmed that the warmest part of the Last Interglacial Period was about 2 °C warmer than the Holocene. Apart from arguments based on modern surface-snow data and on a dynamically simple isotopic model, such an interpretation is consistent with independent approaches such as that based on crystal-size variations and climate modelling.

A special emphasis is given to the comparison between the Vostok and the marine records. The well-marked similarities between, in particular, the estimated ice volume and the Vostok temperature suggests that our record is, at least qualitatively, of relatively large geographical significance. Such agreement is not observed before 110 kyr BP owing to discrepancies between the Vostok and marine chronologies. The duration of the Last Interglacial Period in the Vostok record is about twice as long as expected, if the late Pleistocene Earth's climate was governed only by Northern Hemisphere high-latitude summer insolation. Our present strategy is to keep this Lorius *et al.*[4] timescale, as long as there is no inescapable argument to modify it.

It is remarkable that without any radiometric dates or orbital tuning the Vostok temperature record shows concentration of variance near the obliquity and precession frequency bands, which strongly supports the climatic role of orbital forcing. This aspect and the influence of other forcings (in particular atmospheric CO_2) is examined in the two companion papers[8,37]. These two studies illustrate ways in which obtaining the first continuous long record of the atmospheric temperature, which is the main achievement of the present study, contributes to a better understanding of glacial–interglacial climatic changes.

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Vostok ice core provides 160,000-year record of atmospheric CO_2

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Direct evidence of past atmospheric CO_2 changes has been extended to the past 160,000 years from the Vostok ice core. These changes are most notably an inherent phenomenon of change between glacial and interglacial periods. Besides this major 100,000-year cycle, the CO_2 record seems to exhibit a cyclic change with a period of some 21,000 years.

ALTHOUGH the first direct CO_2 measurements in the atmosphere were made in the second half of the nineteenth century, atmospheric CO_2 variations have been monitored in a systematic and reliable manner only since 1958. Fortunately, nature has been taking continuous samples of the atmosphere at the surface of the ice sheets throughout the ages. This natural sampling process takes place when snow is transformed into ice by sintering at the surface of the melt-free zones of the ice sheets, with air becoming isolated from the surrounding atmosphere in the pores of the newly formed ice. After pore closure the gas remains stored in the ice moving within the ice sheet. During this natural air sampling and storage process, different mechanisms could alter the original atmospheric composition[1,2]. But by choosing appropriate sampling sites, ice cores (for example see ref. 1) and experimental methods[3], past CO_2 changes in the atmosphere can be determined with high confidence by analysing the air enclosed in the pores of the ice.

Previous results from ice-core analysis have already provided important reliable information on the 'pre-industrial' CO_2 level and the recent CO_2 increase induced by anthropogenic activities[4-6]. Striking CO_2 changes have also been detected in this way in the ice record covering the last 30-40 kyr[7-10], including the large CO_2 increase associated with the climatic shift from the Last Glacial Maximum (~18 kyr BP) to the Holocene.

Because of the extremely low temperatures at Vostok (present-day mean annual temperature is −55.5 °C) and the good core quality, the 2,083-m-long ice core recovered by the Soviet Antarctic Expeditions at Vostok (East Antarctica) provides a unique opportunity to extend the ice record of atmospheric CO_2 over the last glacial-interglacial cycle back to the penultimate ice age about 160 kyr ago[11]. Over this timescale a high correlation is found between CO_2 concentrations and Antarctic climate, with significant oscillatory behaviour of CO_2 between high levels during interglacial and low levels during glacial periods. The CO_2 record also seems to exhibit a cyclic change with a period of ~21 kyr, that is, around the orbital period corresponding to the precession.

Experimental procedure

Gas extraction and measurements were performed with the 'Grenoble analytical setup' described by Barnola *et al.*[12]. The method is based on crushing the ice under vacuum without melting, expanding the gas released during the crushing in a pre-evacuated sampling loop, and analysing the CO_2 concentrations by gas chromatography.

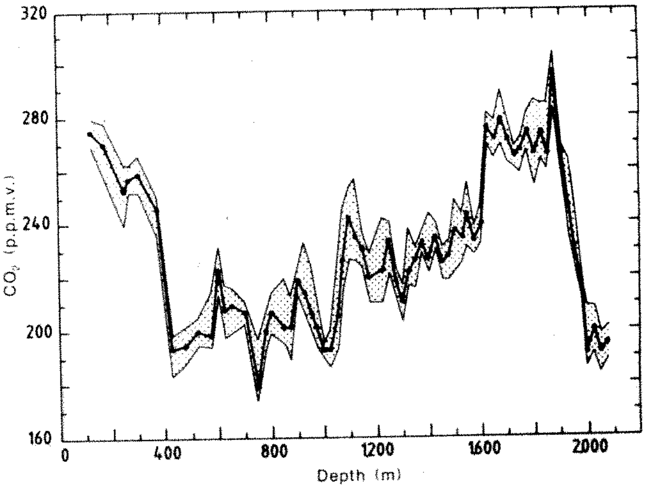

Fig. 1 CO_2 concentrations (p.p.m.v.) plotted against depth in the Vostok ice core. The 'best estimates' of the CO_2 concentrations are indicated by dots and the envelope shown has been plotted taking into account the different uncertainty sources.

The analytical system, except for the stainless steel container in which the ice is crushed, is calibrated for each ice sample measurement with a standard mixture of CO_2 in nitrogen and oxygen. The corresponding accuracy (2σ) is evaluated from the standard deviation of the residuals corresponding to the calibration regression and ranges from 3 to 12 parts per million by volume (p.p.m.v.) for the measurements presented in this article.

We recently discovered an additional error due to our experimental system when a significant amount of water vapour is detected by the gas chromatograph. In such a case, selective CO_2 transport by water vapour (similar to that observed by Neftel *et al.*[4]) back to the ice crushing container is suspected of depleting the CO_2 from the air extracted from the ice and injected

Table 1 Mean CO_2 concentrations in Vostok ice core (best estimates and corresponding uncertainties, against depth and age)

Depth (m)	Age of the ice (yr BP)	Mean age of the air (yr BP)	CO_2 concentration (best estimate) (p.p.m.v.)	Depth (m)	Age of the ice (yr BP)	Mean age of the air (yr BP)	CO_2 concentration (best estimate) (p.p.m.v.)
126.4 (Y)*	4,050	1,700	$274.5\mp^{5}_{5}$	1,274.2 (Y, N)	87,980	84,700	$218.8\mp^{5.5}_{5.5}$
173.1 (N)	5,970	3,530	$270.0\mp^{13}_{8}$	1,299.3 (N)	89,940	86,680	$210.0\mp^{7}_{7}$
250.3 (N, N)	9,320	6,800	$252.0\mp^{13}_{10.5}$	1,322.5 (A)	91,760	88,520	$221.5\mp^{4}_{17}$
266.0 (N)	10,040	7,500	$257.0\mp^{5}_{5}$	1,349.0 (N)	93,860	90,630	$226.0\mp^{16}_{5}$
302.6 (N)	11,870	9,140	$259.0\mp^{7}_{7}$	1,374.8 (Y)	95,910	92,700	$234.0\mp^{4}_{4}$
375.6 (Y)	16,350	12,930	$245.0\mp^{10}_{5}$	1,402.5 (A)	98,130	94,940	$226.5\mp^{5}_{18}$
426.4 (N)	20,330	16,250	$193.0\mp^{10}_{5}$	1,425.5 (Y)	100,000	96,810	$236.0\mp^{5}_{5}$
474.2 (N)	24,280	20,090	$194.5\mp^{7}_{7}$	1,451.5 (N)	102,210	98,950	$225.0\mp^{6}_{6}$
525.1 (Y)	28,530	24,390	$200.0\mp^{5}_{5}$	1,476.1 (N)	104,410	101,040	$229.0\mp^{10}_{5}$
576.0 (A)	32,680	29,720	$198.0\mp^{4}_{17}$	1,499.6 (Y)	106,610	103,130	$238.5\mp^{16}_{11}$
602.3 (Y)	34,770	30,910	$223.0\mp^{9}_{9}$	1,526.3 (N)	109,240	105,620	$234.5\mp^{9}_{9}$
625.6 (N)	36,600	32,800	$207.0\mp^{10}_{10}$	1,547.0 (Y)	111,250	107,650	$244.0\mp^{12}_{12}$
651.6 (Y)	38,600	34,870	$210.0\mp^{11}_{5}$	1,575.2 (N)	113,850	110,510	$233.5\mp^{5}_{4}$
700.3 (N)	42,320	38,660	$207.0\mp^{4}_{4}$	1,598.0 (N)	115,850	112,700	$240.0\mp^{10}_{7}$
748.3 (A)	45,970	42,310	$178.5\mp^{5}_{18}$	1,626.5 (Y)	118,220	115,290	$276.0\mp^{6}_{6}$
775.2 (Y)	48,000	44,350	$200.0\mp^{16}_{5}$	1,651.0 (Y, N)	120,170	117,410	$271.5\mp^{7}_{7}$
800.0 (N, Y, Y)	49,850	46,220	$207.7\mp^{8.4}_{6.6}$	1,676.4 (N)	122,100	119,500	$280.0\mp^{10}_{10}$
852.5 (A)	53,770	50,150	$201.0\mp^{6}_{19}$	1,700.9 (N)	123,900	121,430	$271.0\mp^{8}_{8}$
874.3 (N)	55,450	51,770	$201.0\mp^{12}_{12}$	1,726.8 (N, N)	125,730	123,380	$265.3\mp^{4}_{4}$
902.2 (N)	57,660	53,860	$219.5\mp^{5}_{10}$	1,747.3 (Y, N)	127,150	124,880	$267.0\mp^{8}_{5}$
926.8 (A)	59,670	55,780	$214.5\mp^{6}_{19}$	1,774.1 (N)	129,020	126,770	$275.0\mp^{5}_{7}$
951.9 (A)	61,790	57,800	$206.5\mp^{5}_{18}$	1,802.4 (A)	131,030	128,780	$266.5\mp^{12}_{18}$
975.7 (Y)	63,880	59,770	$201.0\mp^{8}_{8}$	1,825.7 (Y, N)	132,700	130,460	$275.0\mp^{18}_{10}$
1,002.5 (N)	66,230	62,080	$192.0\mp^{3}_{3}$	1,850.5 (A)	134,510	132,280	$266.0\mp^{6}_{19}$
1,023.5 (N)	68,040	63,960	$193.0\mp^{7}_{7}$	1,875.9 (Y)	136,450	134,170	$296.5\mp^{13}_{8}$
1,052.4 (A)	70,470	66,540	$205.5\mp^{12}_{20}$	1,902.0 (Y)	138,660	136,170	$266.0\mp^{4}_{4}$
1,074.8 (A)	72,330	68,490	$226.5\mp^{11}_{19}$	1,928.0 (A)	141,170	138,410	$246.5\mp^{10}_{18}$
1,101.4 (Y)	74,500	70,770	$243.0\mp^{16}_{11}$	1,948.7 (A)	143,440	140,430	$231.0\mp^{7}_{20}$
1,124.2 (A)	76,330	72,690	$235.0\mp^{9}_{22}$	1,975.3 (N)	146,860	143,370	$217.0\mp^{4}_{4}$
1,148.7 (Y)	78,270	74,720	$230.5\mp^{7}_{7}$	1,998.0 (A)	150,330	146,340	$191.0\mp^{5}_{18}$
1,175.0 (N)	80,320	76,860	$219.5\mp^{9}_{9}$	2,025.7 (N)	154,980	150,700	$200.5\mp^{8}_{8}$
1,225.7 (A)	84,220	80,900	$222.5\mp^{12}_{20}$	2,050.3 (N, N)	159,100	154,970	$191.3\mp^{7.5}_{7.5}$
1,251.5 (N)	86,220	82,920	$234.0\mp^{12}_{7}$	2,077.5 (N)	163,670	159,690	$195.5\mp^{6}_{6}$

* (Y) indicates the presence and (N) the absence of an H_2O peak in the chromatographic analysis. Ambiguous cases are denoted (A).

into the gas chromatograph. Experiments indicate that the presence of water vapour in the analysed air is not systematic but, when it occurs, leads to measured CO_2 concentrations lower by about 13 p.p.m.v. than in its absence. For the 75 Vostok core measurements reported in this paper, we found 22 cases with a water-vapour peak and 37 cases without. The presence of this peak was ambiguous for the 16 remaining measurements, leading in those cases to an additional uncertainty. Another source of uncertainty lies in the fact that two different stainless steel containers were used for ice crushing and that the respective results differ statistically by about 5 p.p.m.v.

For each depth level our 'best estimate' of the CO_2 concentration takes into account the correction of +13 p.p.m.v. for the effect of water vapour where there were unambiguous water-vapour peaks. For consistency with previously published data obtained with the same experimental setup[5,10,12] we added, when evaluating this 'best estimate', 5 p.p.m.v. to the CO_2 concentrations measured with the crushing container giving statistically lower values.

All the identified sources of uncertainty (experimental accuracy, effects of water vapour and crushing container) have been taken into account in plotting the CO_2 envelope shown in Fig. 1 and lead to errors around the 'best estimates' ranging from +3 to +22 p.p.m.v. on the positive side and from −3 to −16 p.p.m.v. on the negative side (Table 1). A single measurement was generally performed on each of the 66 depth levels investigated. Eight levels were nevertheless sampled and measured 2 or 3 times. For these levels the scatter of the multiple measurements is generally much smaller than the width of the envelope.

Although we are confident in the relative CO_2 variations measured, a systematic error could affect the absolute values. A recent comparison, still in progress, between results from the Physics Institute of Bern and our laboratory indicates that the values measured in Bern are systematically higher by about 10 p.p.m.v. The results of the comparison, when completed, will be published jointly by the two groups elsewhere.

Vostok CO_2 record

Dating the air found in ice bubbles. Dating the successive ice layers at Vostok has been discussed and an ice chronology established by Lorius *et al.*[11]. This ice chronology cannot, however, be directly applied to the dating of the CO_2 record. Indeed, owing to the air enclosure process in the ice, the air is isolated from the atmosphere in the firn layers well after the precipitation has been deposited at the surface of the ice sheet, and consequently air extracted from the ice is younger than the ice itself.

The following assumptions have been made to date the air in relation to the ice: (1) the air in the firn layers is well mixed with the atmosphere until firn pores close; (2) pore closure occurs roughly in the firn density interval 0.80–0.83 (ref. 13), that is, in the deepest layers of the firn (found between about 86 and 96 m depth at Vostok); and (3) the density–depth profile has remained unchanged over the past 160 kyr.

Based on these assumptions the difference between the age of the ice and the mean age of the air, which is dependent on accumulation rate, is evaluated taking into account the temporal changes of the accumulation rate[11]. This calculated difference varies between about 2,500 yr for the warmest to about 4,300 yr for the coldest periods.

On the other hand, all the air enclosed in an ice sample has not been pinched off from the atmosphere at the same time but rather pore after pore over a time interval which, with the above assumptions, lies between 300 and 750 yr. This acts as a low-pass filter and means that each CO_2 measurement represents roughly an average value over several hundred years.

All the CO_2 ages given in this paper are based on the ice

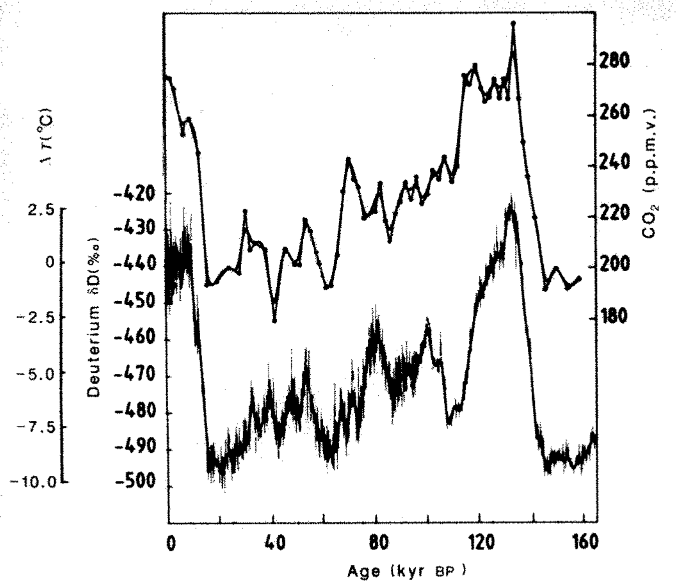

Fig. 2 CO_2 concentrations ('best estimates') and smoothed values (spline function) in p.p.m.v. plotted against age in the Vostok record (upper curves) and atmospheric temperature change derived[14] from the isotopic profile (lower curve). The deuterium scale corresponds to values after correction for deuterium changes of oceanic water[14].

chronology given by Lorius *et al.*[11], and dating of the air relative to the ice was done as described above. No better quantitative method is currently available to evaluate the age of the air. Nevertheless the two first assumptions made above probably lead to a maximum difference between the two ages (air and ice)[5], whereas the last would lead to an age difference too small (by maybe several hundred of years) for the coldest periods.

Description. Sixty-six depth levels were investigated for CO_2 measurements along the 2,083-m core. They are spaced every ~25 m from 850 m depth to the bottom. Because of the presence of fractures in the upper part of the core, the spacing is generally larger above 850 m depth. With this sampling, the mean age difference between two neighbouring levels ranges from ~2,000 to ~4,500 yr.

The record covers the past ~160 kyr and includes the following major climatic periods: the Holocene, the last glaciation, the previous interglacial and the end of the penultimate glaciation[11,14]. Figure 1 shows the results (best estimates and envelope obtained as described above) plotted against depth. Figure 2 shows the CO_2 variations with age of the air.

The CO_2 record exhibits two very large changes, one near the most recent part of the record (~400 m depth or 15 kyr BP) and the other near the earliest part of the record (~1,950 m depth or 140 kyr BP), between two levels centred on 190-200 and 260-280 p.p.m.v. roughly typical of the lowest and highest values of the whole record. The high level is comparable with the so-called 'pre-industrial' CO_2 level that prevailed roughly 200 years ago, just before the large anthropogenic disturbance of the CO_2 cycle; the low level ranges among the lowest values of the known geological history of carbon dioxide over the last 10^8 yr (ref. 15). These two large CO_2 changes correspond to the transitions between full glacial conditions (low CO_2) of the last and penultimate glaciations, and the two major warm periods (high CO_2) of the record: the Holocene and the previous interglacial.

Between these two extremities of the record, that is, over the period covering mainly the last glaciation, the CO_2 shows a general decreasing trend with the lowest values found over the last part of the glaciation (between ~1.025 and 425 m depth, or 65 and 15 kyr) and with two marked breaks: a first decrease of ~40 p.p.m.v. at ~1,610 m depth (~114 kyr BP), and a second of ~50 p.p.m.v. at ~1,070 m depth (~68 kyr BP). Other CO_2 changes are superimposed on the general decreasing trend, suggesting an oscillatory behaviour with an apparent period of ~20 kyr, especially between ~80 and 20 kyr BP. These changes are more pronounced in the last part of the glaciation, but note that some of the variability observed above 850 m depth could be due to the lower ice quality in that part of the core.

The fact that the record does not indicate a continuous long-term trend is an important result in itself regarding possible fractionation of the different gas components due to the interaction with the ice lattice and which should be reflected by such a trend. This adds confidence to the preservation of the atmospheric CO_2 record in the ice over such a long period.

Spectral analysis. The record, as shown in Fig. 2, suggests a cyclic behaviour of the CO_2 changes. Because of the potential link between CO_2 and climate, and consequently between CO_2 and astronomical cyclic forcing of climate, a spectral analysis of the CO_2 record has been done. For comparison with the δD record of Vostok (see below), the same procedure as in ref. 14 has been applied, using values equally spaced every 200 years and obtained by linear interpolation of our best estimates for CO_2 measurements. Because of the short length of the record (relative to the frequencies of interest), we combined different spectral techniques to extract reliable information. Blackman-Tukey (BT) and maximum entropy (ME) methods with and without prewhitening were applied. The BT method has a poor resolution but well defined statistical properties; the use of the ME method increases the frequency resolution but the amplitude estimate has to be interpreted with caution[16]. The prewhitening allows the removal of part of the variance at low frequencies and a more detailed picture in the tilt and precession frequency bands. The ME spectra shown in Fig. 3 were obtained with the Barrodale and Eriksson algorithm[17].

The spectra obtained with the unprewhitened series are dominated by the 100-kyr components (116 kyr for the ME peak). The BT spectrum (not shown in Fig. 3) is relatively flat for higher frequencies but shows a significant bump for periods between 25 and 20 kyr. The ME spectrum indicates a concentration of variance in this frequency band with a well-defined peak at 21 kyr; it also displays a peak at ~55 kyr. Prewhitening, which gives more detailed information for this part of the spectrum, confirms the position of a 21-kyr peak (21.0 kyr for ME). Sensitivity analysis shows the remarkable stability of this peak ($\sigma = 0.1$ kyr) with respect to the lag value (BT) or to the autoregressive order (ME). The ME prewhitened CO_2 spectrum is thus dominated by this component of ~21 kyr and also shows a secondary peak at ~40 kyr (43.1 kyr). As the latter may result from the variance transfer of the 55-kyr peak mentioned above (unprewhitened ME spectrum), it must be interpreted very cautiously.

Because of the relatively small number of measurements with corresponding errors, any interpretation of the spectral analysis needs to be cautious. To check the significance of the spectrum results we have performed some tests with random curves placed within the error band. The results show a rather good stability of the peaks, especially for 21 kyr (20.9 ± 0.2). Some implications of this CO_2 spectrum on the CO_2-climate correlation and on the CO_2 cycle are discussed below.

Comparison with other ice records. Several ice cores from Antarctica and Greenland have already shown CO_2 variations over the past 30-40 kyr (D 10 (ref. 7), Dome C[7,10], Byrd[8] Camp Century[8], Dye 3 (refs 9, 18)). The Vostok profile is in good agreement with the general common features of these records, which show low CO_2 values (~200 p.p.m.v.) during the Last Glacial Maximum (~18 kyr BP) and an increase of the atmospheric CO_2 associated with the glacial-Holocene transition.

Other striking individual CO_2 features are also observed in some of these previous ice records. For instance, abrupt CO_2 changes are recorded in the Dye 3 core between 30 and 40 kyr BP (ref. 9) and in the Dome C core near the end of the glacial-Holocene transition[7,10]. They do not appear in the measured

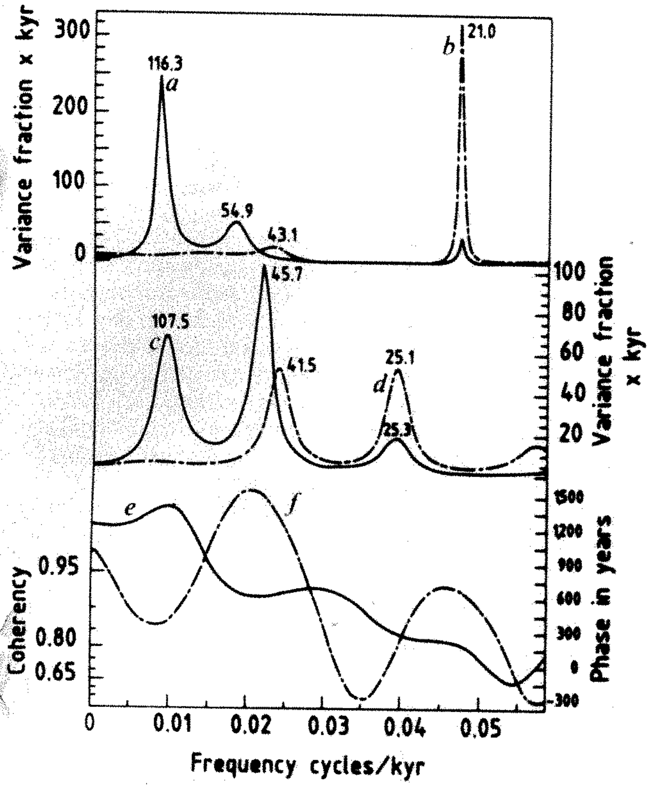

Fig. 3 *a*, *b*, Variance spectra of the Vostok CO_2 record. The normalized variance density is plotted against frequency in cycles per kyr. The continuous line (*a*) refers to the original series and the dashed line (*b*) to the series after prewhitening by a first-order difference filter. The spectra were obtained by the ME method applying the Barrodale and Ericksson algorithm with an autoregressive order of 40%. *c*, *d*, Variance spectra of the Vostok isotope temperature record from ref. 14, obtained with the same procedure. *e*, *f*, Cross-spectral analysis between CO_2 and temperature for coherency (*e*) and phase spectrum (*f*), obtained by applying the Blackman-Tukey method. The phase (right scale) is positive when CO_2 lags the temperature.

Vostok profile, but our sampling does not allow the search for such rapid changes; furthermore, the low-pass filter effect of the air-trapping process (mentioned above) may prevent any detection in the Vostok core of the changes observed in the Dye 3 core. Note also that the high CO_2 level (>270 p.p.m.v.) observed in the Dome C core between 23 and 30 kyr BP is not found in the Vostok record. These high CO_2 values could be due to the poor quality of the Dome C ice in this part of the core[19].

Thus, taking into account the resolution of the different records, the Vostok CO_2 results are consistent with the other ice records already published, covering only the most recent part of the time interval revealed by the Vostok core.

Comparison with deep-sea sediment record. $\delta^{13}C$ differences between planktonic and benthic foraminifera found in a deep-sea core (V 19-30) have been used by Shackleton *et al.*[20] to deduce a record of atmospheric CO_2 covering approximately the last climatic cycle and by Shackleton and Pisias[21] to extend this record over the past 340 kyr. The basic idea, following an approach developed by Broecker[22], is that a $\delta^{13}C$ difference between ocean surface and deep waters is associated with the removal of carbon from the surface by photosynthesis and consequently with the atmospheric CO_2 concentration level. The CO_2 record thus obtained agreed fairly well with the available CO_2-ice record, that is, corresponding to the period covering the past ~40 kyr.

We are now able, with the Vostok ice record, to extend the comparison over the past ~160 kyr. For this comparison we use the more recent data by Shackleton and Pisias[21] for the marine record. Major similarities in the general trends are observed. Both records show rapid and large increases of CO_2 concentrations associated with the two deglaciations and exhibit the highest CO_2 values during the interglacial periods. They also show together a relatively abrupt CO_2 decrease associated with the beginning of the last glaciation.

The comparison is limited in detail because of differences in timescales[14] and in time resolutions between the ice and marine records. There are nevertheless significant differences between the two curves, which include the following observations.

(1) The disparity between previous interglacial and Holocene CO_2 values is much smaller in the Vostok record.

(2) The CO_2 concentrations deduced from the marine record approach Holocene levels several times during the last glaciation, unlike the Vostok record.

(3) The morphology of the Vostok CO_2 curve over the last glaciation (that is, over a large common part of the two records) is definitely not the same as that of the V 19-30 curve. Thus the general CO_2 decrease observed in the Vostok core does not appear in the marine record and the most striking change appears in the Vostok curve as a marked CO_2 decrease (between ~71 and 62 kyr BP in the Vostok timescale) and in the V 19-30 record as a large and sharp CO_2 increase (between ~60 and 55 kyr BP in the V 19-30 timescale).

(4) Spectral analysis has been done[21,23] on the CO_2 record recovered from the marine core study. The V19-30 spectrum exhibits peaks around all the orbital periods (~100 kyr for the Earth's eccentricity, ~41 kyr for the obliquity of the Earth's axis and 23 and 19 kyr for the precession of the equinoxes), whereas the Vostok spectrum shows as mentioned above, aside from the 110 kyr period, a peak at ~21 kyr but nothing clearly corresponding to the obliquity frequency. The comparison is, in fact, not straightforward because the record lengths (~340 kyr for V19-30 and ~160 kyr for Vostok) as well as part of the procedures used for the spectral analysis are different. We have checked the possible influence of these differences by applying equivalent procedures to the Vostok record and to the V19-30 data given in ref. 21, limited to the past 160 kyr. The V19-30 spectrum still shows, in contrast with Vostok, a significant peak around the 40-kyr period.

Thus, although the CO_2 record derived from the carbon isotope differences ($\Delta\delta^{13}C$) between planktonic and benthic foraminifera found in the V19-30 core, as well as the Vostok CO_2 record, indicate that alternations of low and high mean CO_2 concentrations and of glacial and interglacial periods are related, there are significant discrepancies between the changes observed in each of the records. Since the ice record provides *a priori* a more direct and accurate measurement of atmospheric CO_2 changes, these differences suggest that the $\Delta\delta^{13}C$ record of the V19-30 core cannot be interpreted as a pure atmospheric CO_2 curve.

Implications for climate

In this section we compare the CO_2 profile described above with the ice isotopic record obtained on the same core[14] and discuss some climatic implications. A companion paper[24] addresses the potential role of various forcing factors, including CO_2, for producing the temperature record derived from the isotopic composition of the Vostok ice core.

The isotopic record shows the 'dramatic' changes between the two glaciations and the two interglacial periods and provides a detailed description of other climatic variations, especially during the last glacial and the previous interglacial periods.

Our CO_2 profile and the Vostok climatic record (adapted from ref. 14) are plotted together against time in Fig. 2. The climatic significance of the isotopic profile is fully discussed in refs 11 and 14 and high (low) δD values are associated with 'warm' ('cold') temperatures. The temperature scale refers to the atmospheric surface-temperature changes in the Vostok area. The CO_2

and δD records, as obtained from the same ice core, can be directly compared after allowing for the mean age difference between the ice and the air enclosed in the ice (see section on the dating); this age difference has been taken into account in Fig. 2.

The first obvious feature when comparing the two records is the marked similarity in temporal variations. The high correlation ($r^2 = 0.79$) between the linearly interpolated CO_2 curve based on the best estimates and the smoothed δD curve shown in Fig. 2 reflects that generally high (low) CO_2 concentrations correspond to 'warm ('cold') temperatures in the Vostok area. Thus the CO_2 values for the two interglacial periods (mean concentrations of 263 p.p.m.v. for the Holocene and of 272 p.p.m.v. for the previous interglacial period) are much higher than the concentrations measured in the glacial ice (on average 215 p.p.m.v.). Furthermore during the last glaciation, as mentioned above, the mean CO_2 level was higher during the first part (230 p.p.m.v. between 110 and 65 kyr BP) than during the second part (203 p.p.m.v. between 65 and 15 kyr), which also shows colder climatic conditions.

But a more detailed examination of the two records in Fig. 2 indicates differences:

(1) During the previous interglacial period and the transition towards the last glaciation, although both CO_2 and δD seem to peak simultaneously at ~135 kyr BP (note that the CO_2 peak is documented by only one depth level and therefore needs to be confirmed), the CO_2 record shows relatively high and constant values over at least the 10 kyr after the peak (see Fig. 2), whereas the δD curve clearly decreases over the same time interval. A similar effect, although less obvious, seems to appear around 75 kyr BP when the δD curve shows a decreasing trend whereas the average CO_2 apparently remains constant.

(2) The first 'isotopically cold' peak observed during the last glaciation, around 110 kyr BP, has no low-CO_2 companion peak.

The superposition of the CO_2 and δD records also provides information on the comparative timing of the most significant changes in the two signals. The sparse CO_2 sampling and to a smaller extent the uncertainties in the CO_2 measurements and on the age difference between the air trapped in the ice and the ice itself make the determination of a time difference between the two signals of Fig. 2 only possible for differences larger than 3500 yr. Within this uncertainty, the following trends are observed: (1) δD and CO_2 changes appear almost simultaneously when proceeding from glacial to interglacial conditions; (2) as noted above, the situation could be different when proceeding from the previous interglacial to the last glaciation with the CO_2 change clearly lagging behind the δD decrease.

Furthermore information on the comparison between the two time series can be inferred from the spectral analysis. As mentioned above, the CO_2 spectrum reveals a well-defined peak at 21 kyr and, with prewhitening, a relatively weak secondary peak at ~40 kyr that must be interpreted very cautiously. This contrasts (see Fig. 3) with the temperature spectrum deduced from the δD record and given by Jouzel *et al.*[14]. The temperature spectrum does indeed indicate peaks at ~40 and ~20 kyr (that is, which can be associated with the obliquity and precession cycles), with the 40-kyr peak dominating the 20-kyr. The results (Fig. 3) of the cross-spectral analysis for coherence and phase spectrum between CO_2 and temperature (δD) show high coherency in the orbital frequency band and indicate that CO_2 could lag the temperature slightly. This lag is not statistically significant but could reflect, as mentioned above, the temperature signal decreasing before the CO_2 when proceeding from the interglacial to the glacial period.

The above comparison deals with a CO_2 signal that should be of global atmospheric significance and a climatic signal that is *a priori* relevant to Antarctica and consequently to more local conditions. The Vostok δD record shows, in fact, important climatic changes on a worldwide scale[11,14]. In this more general context it is interesting to compare the CO_2 level measured over the previous interglacial with the present atmospheric CO_2 concentrations, because this period of the past has been proposed as an analogue for a future CO_2-warmed world. The Vostok results indicate a mean CO_2 concentration (272 p.p.m.v.) comparable with the 'pre-industrial' CO_2 level (estimated to be 275 ± 10 p.p.m.v. (ref. 25)). Furthermore, the CO_2 peak of about 300 p.p.m.v. found at ~135 kyr BP, if confirmed, is significantly lower than the current level of about 345 p.p.m.v.

The high correlation between atmospheric CO_2 and isotopic temperature found in the Vostok record suggests that the radiative effect of CO_2 coupled with associated feedback mechanisms appears to be a serious candidate as one of the driving forces behind the palaeo-temperature changes recorded in the Vostok core. The relative weights of different types of forcing in the reconstruction of the Vostok temperature variations are discussed in the companion paper[24]. This approach points out the probable importance of CO_2 in influencing the main feature of the climate during the last climatic cycle.

Examining the parts of the CO_2 and δD records containing the most pronounced changes, although the two transitions from glacial to interglacial conditions shown by the record indicate almost simultaneous changes for CO_2 and isotopic temperature, the situation is different when looking at the transition between the previous interglacial period and the last glaciation. As shown in Fig. 2, a large portion, about 7 °C, of the isotopic temperature decrease leading to the glacial conditions takes place between ~132 and 115 kyr, a time during which no significant CO_2 changes are observed. Our results thus indicate that climatic forcings other than CO_2 induced a large part of the temperature change over Antarctica linked with the interglacial-glacial transition. The marked CO_2 decrease (from ~275 to 235 p.p.m.v.) appears only during the final phase of the climatic cooling (between ~115 and 110.5 kyr) and is associated with a decrease of ~2 °C in the Vostok isotopic temperature.

Based on the above discussion, we suggest that the interactions between climate and CO_2 could be different depending on whether the climate shifts from glacial to interglacial or from interglacial to glacial conditions. In the latter case, orbital and orbitally derived forcing could have influenced the marked cooling trend in the Antarctic with a dominant contribution to the temperature decrease, before the potential climatic effect of the change in CO_2 concentrations. Note that the second (in terms of amplitude) cooling trend, found around 75 kyr BP in the Vostok record, exhibits a similar behaviour of the CO_2-δD comparative variation.

Implications for CO_2 cycle

As well as providing information on the CO_2-climate interaction, our CO_2 profile allows us to discuss the modifications of the carbon cycle during the last climatic cycle. Based on previous ice core results over the past 30-40 kyr, several models have been proposed to explain the increase of atmospheric CO_2 associated with the last deglaciation. Detailed descriptions of these models can be found in Broecker and Peng[26] or Berger and Keir[27]. These models are based on the idea that the biological productivity of the ocean controls the atmospheric CO_2 but they differ in the mechanisms driving these productivity variations.

In one family of models, sea-level variations are responsible for the atmospheric CO_2 changes[22,28]. The general trend of the Vostok CO_2 profile is similar to the benthic $\delta^{18}O$ records, which mainly reflect ice volume or sea level (for example see Fig. 2*b*, *e* of the companion paper[24]), making these models attractive. Berger and Keir[27] and Keir and Berger[29] attempt to model the past atmospheric CO_2 modifications due to sea level variations. Choosing appropriate parameters, the morphology of the derived CO_2 curve is close to that of the Vostok curve and the CO_2 variations lag the sea-level changes by ~1 kyr. To test this time lag, it is necessary first to evaluate the phase relationship between the CO_2 and the Vostok temperature and secondly between the Vostok temperature and the sea level. In deglaciations, as already mentioned, the CO_2 begins to increase almost simultaneously with the Vostok temperature. The precise timing between the temperature and the sea level is difficult to obtain because these two signals are not recorded in the same medium. It is nevertheless reasonable to assume that the sea level cannot increase before the Antarctic temperature, as suggested by the interpretation of the marine sediment record, which indicates that changes in Southern Hemisphere sea-surface temperature have generally led the changes in ice volume[30,32]. Although denser sampling would be required for a more definite conclusion, the Vostok results thus suggest that at the end of glaciations the CO_2 increased before the sea level and not after as predicted by the models mentioned. At the beginning of the last glaciation (~120 kyr BP), the CO_2 decreased ~10 kyr after the temperature and thus most probably after the sea-level change. Although this phase relation cannot exclude the influence of the sea level on the CO_2 variations during this period, it is important to note that the CO_2 decrease is very sharp (35 p.p.m.v. in 2 kyr) and seems incompatible with the mechanisms involved in the models proposed. We suggest that this abrupt change of the atmospheric CO_2 between two nearly constant values could be due to an abrupt modification of the deep oceanic circulation possibly linked with the sea-level decrease. Such rather rapid circulation changes associated with climatic transitions have been shown to occur[33].

In another family of models, changes in oceanic circulation affect the biological productivity of the surface ocean and thus the atmospheric CO_2 concentration. These models stress changes occurring in the high latitude deep-water formation areas[34-36] and in the upwelling zones at low latitudes[35]. The spectral analysis of the Vostok CO_2 record shows, as well as the 110-kyr component, a very weak and doubtful 41-kyr and a clear 21-kyr peak. This feature can give some indications of the latitudes involved in the forcing of the atmospheric CO_2. The latitudinal variation of the insolation spectra is complex. Although the presence of an obliquity component (41 kyr) seems to be a characteristic of high latitudes, this component is negligible around the equinoxes and becomes important around the solstices[37]. From marine proxy data, the spectral analysis of the sea-surface temperature of the North Atlantic shows that, apart from the 110-kyr component, the 41-kyr component is dominant for latitudes greater than 45° N (ref. 38), and we can note that the Vostok temperature profile also exhibits a strong 41-kyr period[14]. The quasi-absence of a 41-kyr period in the CO_2 record could thus suggest that the high latitudes do not have a major influence on the CO_2 variations. Nevertheless we cannot exclude an influence of these latitudes during part of the year, in particular around the equinoxes.

At low latitudes, upwelling areas are known to play a key role in the total biological productivity of the oceans and may thus influence the atmospheric CO_2 significantly. Evidence exists from the reconstructed sea-surface temperatures that upwellings were stronger during the Last Glacial Maximum (18 kyr BP)[39]. Similar conclusions are obtained from the palaeoreconstructions of trade-wind strength[40-42], responsible for the upwelling currents. Some of these indicators exhibit similarities with the atmospheric CO_2, for instance at the end of the two last glaciations the upwelling productivity off the NW African coast decreased before the increase in sea level[40], as did the winter trade-wind speed near the Peru coast[41]. Note also that the upwelling productivity off NW Africa was high during only the second part of the glaciation, that is, between ~70 and 15 kyr BP[41], and that the Vostok aluminium concentration profile exhibits three very pronounced spikes suggesting stronger atmospheric circulation at ~150, ~70 and ~20 kyr BP[43]. These spikes correspond to very low CO_2 values (~200 p.p.m.v.).

All these facts suggest that atmospheric circulation, coupled with oceanic circulation, at least through the upwelling currents, could have played an important role in past CO_2 variations. This influence may have been especially important between 70 and 15 kyr BP and thus could be responsible for at least part of the lower CO_2 values observed during this period compared with the values recorded during the previous part of the glaciation (110-70 kyr BP).

In summary, it seems that the atmospheric CO_2 variations recorded in the Vostok core could be explained by two kinds of changes in the oceanic circulation, that is, the deep changes possibly driven by sea level and the surface changes driven by atmospheric circulation. In this case only deep circulation changes could be the cause of the CO_2 variations between the last interglacial and the first part of the glaciation (110-70 kyr), whereas both deep and surface circulation could be responsible for the lower CO_2 values found during the second part of the glaciation (70-15 kyr) relative to the first part. During glacial-interglacial transitions, the CO_2 increase could be initiated by surface circulation changes relayed by the deep circulation changes associated with the sea-level increase.

Conclusions

An atmospheric CO_2 record over the past 160 kyr has been obtained from the Vostok ice core. This CO_2 record is probably the purest available, covering the last climatic cycle. The CO_2 changes thus revealed, which are of global significance, are well correlated with the Antarctic temperature record derived from the ice isotopic profile measured on the same core. Such a high correlation would be expected if CO_2 plays an important role in forcing the climate.

The results also suggest a different behaviour of the relative timing between CO_2 and Antarctic climate changes depending on whether we proceed from a glacial to an interglacial period or vice versa. This may have implications concerning the relation between cause and effect inside the CO_2-climate system, but more detailed measurements in the transition parts of the record are required before firm conclusions can be drawn. Long-term CO_2 changes are dominated by marked glacial-interglacial oscillations between ~190-200 and 260-280 p.p.m.v. A period of ~20 kyr similar to the orbital precession period, appears in the decreasing CO_2 trend covering most of the last glaciation and is supported by spectral analysis. These CO_2 changes could be linked with oceanic circulation changes but a more precise interpretation would in particular require the determination of the $\delta^{13}C$ of the CO_2 extracted from the air bubbles trapped in the ice and a comparable chronology for marine and ice core records.

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Vostok ice core: climatic response to CO_2 and orbital forcing changes over the last climatic cycle

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Vostok climate and CO_2 records suggest that CO_2 changes have had an important climatic role during the late Pleistocene in amplifying the relatively weak orbital forcing. The existence of the 100-kyr cycle and the synchronism between Northern and Southern Hemisphere climates may have their origin in the large glacial-interglacial CO_2 changes.

FROM the Vostok Antarctic ice core drilled by the Soviet Antarctic Expeditions, we have, in two companion articles[1,2], derived time series of the central East Antarctic atmosphere temperature and of atmospheric CO_2 content spanning the past 160 kyr. These studies suggest that over this period the climate at Vostok was, at least in part, driven both by the variations in atmospheric CO_2 and by orbital forcing which would be exerted through insolation changes in the Northern Hemisphere and locally[3]. Here we examine the relative influence of these various forcings on a quantitative basis by calculating the temperature signal as a response to CO_2 and forcings in the Northern Hemisphere and locally. Our simple model can explain $>90\%$ of the climate variance at Vostok. It suggests that CO_2 plays an important role in influencing the Earth's climate during the late Pleistocene.

For two decades the Milankovitch hypothesis[4] that the great Northern Hemisphere ice sheets are controlled by the seasonal contrast of insolation at $\sim 65°$ N has received support, particularly from a thorough analysis of the $\delta^{18}O$ deep-sea record[5,6]. But the implied large amplification of this relatively weak forcing (the total insolation received by the planet varied by $<0.6\%$ over the past 10^6 yr), the observed dominant 100-kyr cycle and the synchronism between the termination of major glaciations in the Northern and Southern Hemispheres do not fit this hypothesis well[7–12]. Further developments of the theory of Pleistocene climates have included the nonlinear response of ice sheets to this orbital forcing[8,13–17] and the role of oceanic circulation[11,18,19], ice-shelf destruction[20,21] and sea-level changes[9,21]. More recently the glacial-interglacial CO_2 changes shown from measurements in air trapped in ancient ice[22,23] and later indirectly derived from $\delta^{13}C$ deep-sea core data[24,25], have focused attention on the possible contribution of the CO_2 greenhouse effect to these major climatic changes[10–12,17,25–28].

To address the problem of glacial-interglacial climatic changes, the Vostok ice-core results are important because they give access in the same core to: (1) a continuous isotope temperature series which is, at least qualitatively, of relatively large geographical significance[1] and (2) a direct estimate of the atmospheric CO_2 content which is global in character[2]. These two series are dominated by a clear ~ 100 kyr signal. They show concentration of variance near orbital frequencies and suggest that there is an interaction between CO_2, orbital forcing and climate on the timescale of glacial-interglacial episodes. Because all the parts of the climatic system (atmosphere, ocean, cryosphere, biosphere) are involved, such interactions are complex. Considering this complexity, then, we shall limit our present purpose to giving and discussing the results of a multivariate analysis between a single climatic output (the Vostok isotope temperature record, ΔT_s, shown in Fig. 1*a*) and multiple climatic inputs. In essence we perform a statistical analysis without specifically accounting for the physical mechanisms involved. We consider three types of climatic forcings: (1) a Southern Hemisphere input, (2) a Northern Hemisphere input, and (3) a CO_2 input.

Methods

These climatic forcings were chosen to cover as far as possible the full range of hypotheses currently proposed in this field. Figure 1 shows corresponding time series, the insolation curves being calculated using the Berger algorithms[29].

As a Southern Hemisphere (SH) input we used either the local insolation (78 °S) received during the entire year (s_1) or the mean daily November insolation at 60 °S (s_2). As mentioned in ref. 3, s_1 shows visual similarities to the Vostok isotope record, which suggests there is a link between ΔT_s and this annual insolation; s_2 is thought to play in important role in the sea-ice extent around Antarctica[30]. In all of the cases considered, the

Vostok temperature is assumed to be in phase with the SH forcing. Additional tests were made using the mid-December insolation at 78° S; the results are quite similar to those produced with s_2. We also attempted to use the solar radiation absorbed at the snow surface ($s_1(1-\alpha)$, where α is the albedo) accounting for the dependence of α on the solar elevation[31]. Although this effect is relatively important at high latitutides, on a seasonal basis, there is no significant modification of the shape of the local forcing in the long term. These two additional approaches were not explored further.

The Northern Hemisphere (NH) component was taken to be either the July insolation at 65° N (n_1), the Imbrie and Imbrie[8] ice volume theoretically derived from this insolation curve (n_2), or the $\delta^{18}O$ reconstructed ice volume (n_3) of Martinson *et al.*[32]. n_1 and n_2 are alternative ways of examining the Milankovitch hypothesis; n_3 is a more direct estimate of the ice volume change. The ice volume change is important as it may be the link between the climates in the two hemispheres, for example through oceanic circulation or sea-level changes. This NH forcing is not expected to be in phase with the SH climatic response[9,10]. We introduced an open parameter, $\Delta\phi$, representing the shift between the NH input and the Vostok climatic output (equation (2)). There is no significant influence of $\Delta\phi$ on the result of the multivariate analysis. All the results given in Table 1 correspond to $\Delta\phi = 0$.

Our implicit assumption about the CO_2 input is that the changes in the CO_2 content of the atmosphere are part of climatic forcing. Two key ideas to keep in mind in the final assessment of the results of our statistical analysis are that CO_2 changes might just be a consequence of climatic change without much effect on the climatic change itself and are possibly triggered by orbital forcing, as suggested by the presence of a precessional component in the CO_2 record[2].

The radiative forcing associated with the CO_2 variations was evaluated in terms of its direct temperature effect (without any climatic feedback) from the empirical formula computed by Hansen *et al.*[33] which fits, to better than 1%, the results of a radiation scheme of a three-dimensional model[34] for CO_2 between 100 and 1,000 p.p.m.v. With respect to the Holocene temperature, it yields

$$\Delta T_{CO_2} = \ln(1 + 1.2\,X + 0.005\,X^2 + 1.4\times10^{-6}\,X^3) - 6.64 \quad (1)$$

X being the atmospheric CO_2 concentration in p.p.m.v. (as recorded in the Vostok core). This calculated temperature change without feedbacks, ΔT_{CO_2}, is given in Fig. 1*b*, showing a total amplitude of ~0.6 °C. Owing to oceanic thermal inertia, there is a delay in the climatic response to a CO_2 change but by no more than ~100 yr[34,35]. It is not necessary to account for this delay, which is less than the period over which the atmospheric CO_2 signal is smoothed by the air-trapping process in the ice and far less than the average CO_2 sampling interval[2].

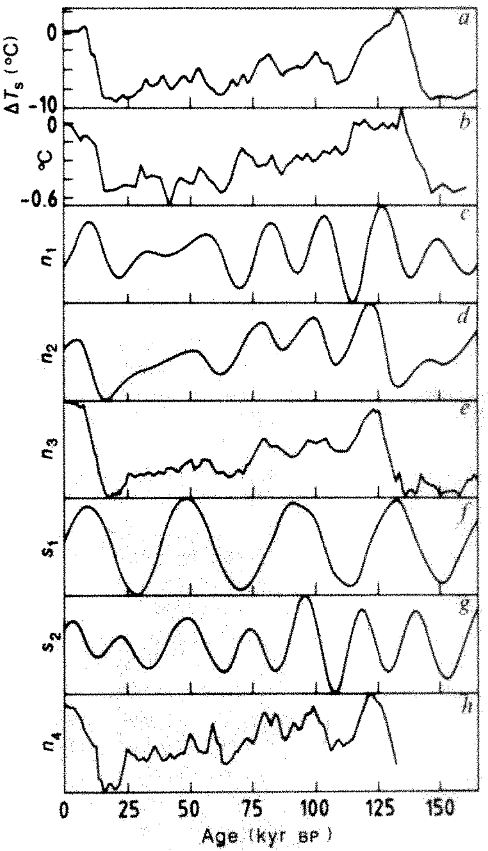

Fig. 1 Time series of the climatic inputs and of the output in the Vostok record. *a*, Vostok isotope temperature change, ΔT_s (Lorius *et al.*[3] chronology); *b*, Vostok atmospheric CO_2 expressed as direct radiative forcing; *c*, insolation, July 65° N; *d*, ice volume change from the Imbrie and Imbrie model; *e*, estimated ice volume change from deep sea core $\delta^{18}O$; *f*, total insolation at 78° S during the entire year; *g*, insolation, November, 60° S; *h*, estimated ice-volume change from Labeyrie *et al.*[38]. Curves *c*–*h* are scaled so as to have about the same amplitude.

We applied the numerical procedure described in Jenkins and Watts[36] writing the climatic output, ΔT_s, as

$$\Delta T_s(t) - \overline{\Delta T_s} = a_{CO_2}(\Delta T_{CO_2}(t) - \overline{\Delta T_{CO_2}}) + b(s_i(t) - \overline{s_i}) + c(n_i(t-\phi) - \overline{n_i}) + z(t) \quad (2)$$

Table 1 Results of the multivariate analysis

	Case	Output	SH	NH	r^2	a_{CO_2}	w_{CO_2} (%)	w_{SH} (%)	w_{NH} (%)
Lorius *et al.*[3] chronology	1	ΔT_s	s_1	n_1	0.90	13.5	78	16	6
	2	ΔT_s	s_1	n_2	0.88	13.3	79	20	1
	3	ΔT_s	s_1	n_3	0.89	12.7	75	20	5
	4	ΔT_s	s_2	n_1	0.86	14.0	85	3	12
Redated Vostok series	5	ΔT_{s1}	s_1	n_1	0.90	13.7	79	8	13
	6	ΔT_{s1}	s_1	n_2	0.87	12.5	75	13	12
	7	ΔT_{s1}	s_1	n_3	0.90	9.4	55	11	34
	8	ΔT_{s2}	s_1	n_3	0.92	6.2	35	3	62
0–110-kyr period	9	ΔT_{s3}	s_1	n_1	0.85	12.7	75	13	12
	10	ΔT_{s3}	s_1	n_2	0.84	11.9	71	16	13
	11	ΔT_{s3}	s_1	n_3	0.92	4.9	27	9	64
	12	ΔT_{s3}	s_1	n_4	0.84	11.0	66	15	19

The climatic output at Vostok (temperature change) is given in column 2 and the chosen Southern and Northern Hemisphere inputs in columns 3 and 4 (see text); r^2 is the square of the correlation between ΔT (the calculated temperature change at Vostok) and the output at Vostok; a_{CO_2} is the calculated amplification of the CO_2 temperature effect. The relative weights, w, of each contribution (columns 7–9) represent the percentage of variance explained by each of the three inputs.

where t is the time, the variables with an overbar represent the means of each time series, and s_i and n_i are the SH and NH inputs discussed above. The first three terms on the right of equation (2) correspond to the temperature change that may be attributed to each of the three inputs, and $z(t)$ is a noise term. This equation is solved in such a way to minimize $z(t)$ in a least-square sense using resampled series with equally spaced points every 100 yr. Coarser sampling or the use of slightly smoothed curves for data series were examined but they do not affect the results significantly. The best estimate of ΔT_s, from the three climatic inputs, $\Delta T_s(t) - z(t)$, will be referred to as the 'calculated' Vostok temperature change and denoted by ΔT.

The term a_{CO_2} is the ratio of the temperature change that may be attributed to CO_2, to the direct radiative effect of CO_2 changes (without feedback); it will be referred to as the CO_2 amplification factor. This definition parallels that of the climate feedback factor, f, evaluated from climate model simulations as the ratio of the overall predicted temperature change to the ΔT_{CO_2} without feedbacks. Note that the precision of the determination of a_{CO_2} is no better than that of ΔT_s, that is, ~20% as discussed in ref. 1. Comparison of the amplification (multivariate analysis) and feedback (climate modelling) factors will be useful in the discussion of the CO_2 climate contribution; such a comparison is not greatly affected by this 20% uncertainty. Unlike a_{CO_2}, b and c are not directly expressed in term of temperature changes.

This linear approach is admittedly simplistic because there are certainly nonlinear interactions between the different parts of the climatic system[8,14-17]. Owing to our strategy not to attach the Vostok dating to other palaeoclimatic or forcing series, the use of a more elaborate gain and phase model[36,37] is not justified. However, the influence of various redatings for the period before 110 kyr BP, over which the Vostok and marine chronologies noticeably disagree[1], was also examined. We see that despite its simplicity, this approach provides interesting results about the relative contributions of various forcings. These results are discussed with regard to the 12 cases listed in Table 1. Cases 1-4 refer to the Lorius *et al.*[3] chronology, cases 5-8 to redated Vostok series as described below, and cases 9-12 to the 0-110-kyr period.

Results

Cases 1–4. The first three cases test the NH forcings, keeping the SH input fixed (78° S annual insolation). The temperature output and the CO_2 forcing are those of Fig. 1*a*, *b* with the Lorius *et al.*[3] Vostok chronology based on a glaciological model. The best correlation ($r^2 = 0.90$) is obtained with the July insolation at 65° N. The use of an ice-volume forcing, either calculated (n_2) or reconstructed (n_3) does not improve this correlation. In cases 1-3 the SH contribution (s_1) is significant (up to 20%). This contribution practically disappears if another SH input is used, either s_2 (November, 60° S) as illustrated by case 4 or the mid-December local insolation (not listed). The common feature of all these analyses is that the main contribution comes from CO_2 with a very high associated amplification (~13). This is not surprising as the ΔT_{CO_2} input is already well correlated with ΔT_s ($r^2 = 0.78$). There is, however, an interesting contribution of the other forcings as illustrated in Fig. 2, which shows ΔT and its spectrum along with the same curves for ΔT_s. The NH forcing (July, 65° N) clearly participates in simulating the 110-kyr BP temperature minimum, and the SH forcing (local forcing) brings power in the ~40-kyr frequency band, two important features of the ΔT_s record missing in the CO_2 input[1,2].

Cases 5–8. One possible reason for the relatively low contribution of the orbital input to ΔT (the sum of NH and SH contributions never exceeds 25% in cases 1-4) is that the Vostok core is dated independently of such forcings. On the other hand, there is no significant uncertainty in the relative timing of ΔT_s and CO_2 changes. But we believe that the duration of the last interglacial period was about the same in the Vostok atmospheric record as in the marine records[1]. An inevitable consequence is that one, at least, of the Vostok and deep-sea core datings has to be modified before ~110 kyr BP. We examined the influence of the chronology on our multivariate analysis in testing various redatings.

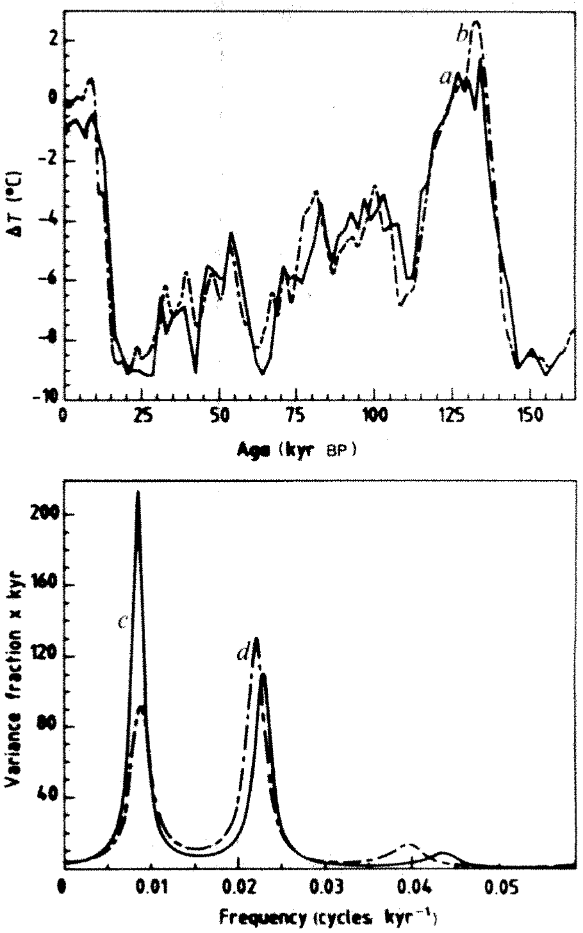

Fig. 2 Top, the calculated temperature (ΔT) obtained with the climatic inputs of case 1 (*a*, continuous line) plotted with the Vostok isotope temperature of ref. 1 (*b*, ΔT_s, broken line). Bottom, the variance spectra of these two curves. The normalized variance density is plotted against frequency in cycles per kyr on linear scales: *c*, ΔT; *d*, ΔT_s. These spectra were obtained by the Maximum Entropy method, applying the Barrodale and Ericksson[42] algorithm with an autoregressive order of 40%.

Cases 5–7 refer to a Vostok timescale modified in such a way as to put the Vostok temperature roughly in phase with that of the sea surface around Antarctica (ΔT_{s1}) taken from RC11-120 (ref. 32). The CO_2 amplification does not significantly decrease ($a_{CO_2} \approx 13$) with either July at 65° N (case 5) or Imbrie ice volume (case 6) as NH input. This is not surprising as <35% of the temperature variance is directly explained by this type of NH forcing. On the other hand, this amplification drops with the reconstructed ice volume (case 7) down to ~9.

This lowering is further accentuated if the ice volume is put in phase with the Vostok record before 110 kyr BP (ΔT_{s2}, case 8, $a_{CO_2} = 6.2$). In case 8, the explained variance is also >0.90 ($r^2 = 0.92$) but now with a predominant contribution of the NH forcing (63% compared with 35% for CO_2), reflecting the very high correlation ($r^2 = 0.88$) observed between the deep-sea core $\delta^{18}O$ curve (n_3) and the redated Vostok temperature (ΔT_{s2}). Such a high correlation could at least partly result from the fact that the ice volume and the Antarctic temperature respond in the same way to the same common forcing and not from a direct influence of ice-volume change on the Vostok temperature. This would artificially increase the contribution of the NH component in our analysis. Consequently, the CO_2 amplification inferred from case 8 should be considered a lower limit for this parameter.

This general picture given by cases 5-8 is not greatly affected if a slightly different Vostok timescale is used (for the period before 110 kyr BP) or if it is the deep-sea record that is fitted in phase with the Vostok series as dated by Lorius *et al.*[3].

Cases 9–12. The last set of results deal with the period since 110 kyr BP (ΔT_{s3}). This shortening of the records lowers the accuracy of the analysis but has the advantage of eliminating many of the problems created by the apparent dating discrepancy between the ice and deep-sea records. This approach essentially supports the results obtained with redated series, but with a further lowering of the CO_2 amplification ($a_{CO_2} = 4.9$) with the Martinson *et al.* series[32] as the NH input (case 11). There is still a relatively low NH contribution (and high CO_2 amplifications) with orbital forcing (cases 9 and 10).

For this period, we also used the ice-volume change recently estimated by Labeyrie *et al.*[38] (Fig. 1*h*, n_4). This results (case 12) in a relatively low influence of this NH input (19%) and intermediate CO_2 amplification (11.0). The difference from case 11 reflects the lower ice-volume–Vostok-temperature correlation obtained with this estimate than with that of Martinson *et al.* ($r^2 = 0.59$ instead of 0.88). It indirectly confirms that part of the Martinson *et al.* signal could originate with temperature, explaining its high correlation with the Vostok record.

Discussion

Again we are aware of the limitations of our multivariate analysis, some of them emerging from the above discussion. First, it may be useful to explore a wider range of possible forcings and associated feedbacks. For example, one can consider, the introduction of different insolation curves or of different model-derived[14,15] or reconstructed ice volume. Effects such as the change in the atmospheric optical depth resulting from variations in aerosol loading[39] or in the concentration of trace gases[40] may affect the climate. We shall note that such parameters are[41] or will be determined from the Vostok ice. In addition it is very important to get accurate absolute dating of the palaeoclimatic series or, at least, to correlate the deep-sea and ice records directly[1].

On the other hand, within these limits, our study allows us to suggest some conclusions about the climatic contribution of various forcings as inferred from our statistical analysis. They can be summed up as follows.

(1) The SH influence is maximum (20%) if assumed to be linked with the local annual insolation; this influence decreases if the record is redated before 110 kyr BP (13% in case 6).

(2) The NH contribution is different if exerted through an orbital (n_1) or orbitally derived (n_2) input or through the $\delta^{18}O$ ice-volume record. Only in this last case (n_3) does the NH relative contribution become predominant ($\leqslant 64\%$).

(3) The relative contribution of CO_2 is generally >50% and as high as 84%. CO_2 contributions <50% are, however, observed when the reconstructed ice volume of Martinson *et al.* is used as an NH input (cases 8 and 11).

In the remaining part of this discussion we focus on how these results affect our current understanding of the Earth's climatic system.

The influence of the local annual insolation was examined in ref. 3. The relative range of variation of this parameter is ~7%, which would have, through direct radiative forcing[34,43], an effect of ~4 °C on the local equilibrium temperature ($\Delta T_e / T_e = \frac{1}{4}(\Delta s_1 / s_1)$, with T_e in K). The amplitude of the SH contribution inferred from our analysis is lower (it never exceeds ~3° C) but still compatible with our working hypothesis of a potential role of this local insolation. Note also that, even though never contributing $\geqslant 20\%$ in the ΔT reconstruction (Table 1), this local input plays a key role in bringing power in the obliquity band, which clearly dominates the Vostok temperature record[1]. There is not, as might have been expected[30], a relation between the extension of sea-ice (if governed by the 60° S November insolation) and the temperature in central Antarctica.

Experiments with general circulation models (GCMs) show that the Northern Hemisphere ice sheets have little influence on atmospheric and sea surface temperature (SST) in the Southern Hemisphere[11,12]. Thus the atmosphere cannot constitute the suggested link between a NH input (65° N insolation or ice volume) and the Vostok climate. Such an interhemispheric link would instead be through a change in the deep oceanic circulation[10,11,18–20]. A proposed mechanism[19] is that under glacial conditions the formation of relatively warm North Atlantic deep water and its injection into circumpolar Antarctic regions are greatly reduced, thereby reducing the SH ocean temperature. Such changes in deep ocean circulation are now well documented all over the last climatic cycle[38,44–48] and are in general agreement with this picture of a predominant role of the rate of formation of North Atlantic deep water. Sea-level variations associated with NH ice-sheet growth and retreat are also often cited as a potential link between NH and SH climates. We note that the suggestion that the global ice-sheet system interlocked by sea level (which transforms regional Milankovitch insolation into a global climate signal) is probably flawed with respect to the Southern Hemisphere[21].

As noted above, no clear information about the phasing of the SH and NH climates, and thus about the potential link between the two hemispheres, can be extracted from our analysis. But it is of interest to examine whether such a specific climatic event as the Younger Dryas revearsal thought to be typical of the NH climate[49] has a SH counterpart. As discussed in ref. 1, the amplitude of this cold reversal is much weaker in the Antarctic cores than in the Greenland cores, suggesting[18,50] that it is a damped response of the SH climate to an initial Northern Atlantic cooling, hypothesized as cause of the Younger Dryas[49]. If the present dating, which puts the Vostok cooling ~1 kyr earlier than the Younger Dryas (dated from 11 to 10.2 kyr BP), seems to contradict this suggestion, we are convinced that this event (unless related to CO_2 atmospheric content variation) is the manifestation of the NH–SH climatic link.

Our discussion of the contribution of CO_2 to climate is based on climate-sensitivity experiments made with GCMs either for modern[33,34] or Last Glacial Maximum (LGM) boundary conditions[11,12,51,52]. As noted above, such experiments allow us to study the various feedback processes influencing the climate sensitivity and to evaluate the net feedback factor, *f*, associated with a given change in radiative forcing. We examine whether the existence of such feedbacks may explain the high-CO_2 amplifications (~5 to ~14) found in this study.

The presentation of the GISS (Goddard Institute for Space Studies) model results[33,34] is particularly well adapted to our purpose of examining feedback processes. With this model the predicted warming for $2 \times CO_2$ experiments is 3 ± 1.5 °C, which corresponds to a global-scale climate feedback of 2.4 ± 1.2. A feature common to most of the GCM simulations is that they show greater changes in high latitudes than in low latitudes, linked to the variations in the amount of sea ice and extent of snow cover. This polar amplification was given as a possible reason for a significant impact in polar regions of the LGM decrease in atmospheric CO_2 content[26]. But the temperature change predicted by the GISS model for modern $2 \times CO_2$ experiments over central Antarctica is quite comparable with that predicted for the entire planet, showing no polar amplification in that region. Such a result does not support the idea that the high CO_2 feedback at Vostok inferred from our statistical analysis may be attributed only to polar amplification.

A more plausible explanation stems from the higher climate sensitivity during glacial than during interglacial periods, a point well documented by GCM models[34,51,52]. The basic reason is that experiments with modern boundary conditions account only for fast feedback processes (water vapour, clouds and sea ice) and not for slow feedback processes such as those associated with the larger extent of sea ice and land ice during glacial periods. As noted by Hansen *et al.*[34], if strong positive feedbacks exist, a moderate additional feedback may cause a large increase in the overall feedback factor. We can calculate the overall feedback from the property of additivity of the associated gains, $g = 1-(1/f)$. From the results of Hansen *et al.* it follows that combining the land-ice feedback ($f = 1.25$) and that due to the additional sea ice ($f = 1.08$) with that resulting from the fast processes ($f = 2.4$) leads to an overall feedback of 7.

The same approach may be followed from the recent stimulations of the LGM by Broccoli and Manabe[12] using the GFDL (Geophysical Fluid Dynamics Laboratory) model. Their experiments with a CO_2 decrease from 300 to 200 p.p.m.v. show a global temperature reduction of 1.2 °C but, in that case, the polar amplification affects all the Antarctic continent over which the temperature decrease is >2 °C (ref. 12). Indeed, the decrease in temperature due to the reduction of atmospheric CO_2 is responsible for almost all of the simulated LGM Southern Hemisphere cooling[12]. The resulting feedback factor is >3 all over Antarctica (ΔT_{CO_2} is calculated as in ref. 34). In their experiments Manabe and Broccoli used LGM continental ice distribution so that the land-ice feedback was already included, but this may be counterbalanced by the fact that the effects of cloud feedback are not included. A moderate cloud feedback ($f = 1.15$, compared with 1.3 in the GISS model[34]) and that due to additional sea ice (assumed to be 1.08) would also lead to a net feedback factor 7, at least over Antarctica.

These estimations do not include vegetation feedback (mentioned in ref. 34) and that postulated by Manabe and Bryan[51] and Denton *et al.*[21] involving respectively thermohaline circulation and floating shelf ice around Antarctica. It thus appears that, over long timescales, high CO_2 feedbacks as deduced from Vostok data are not unreasonable.

In that respect, on a global scale the direct radiative effect due to CO_2 changes (amplitude of ~0.6 °C) is about three times that due to insolation changes (the energy received by the planet varies by ~0.3% over the past 160 kyr, which would account for 0.2 °C). This supports our conclusion from multivariate analysis that CO_2 changes play a more important role than orbital variations. But the presence of the precession frequency in the CO_2 record[2] suggests that orbital forcing is also implicity present in the CO_2 forcing.

Conclusion

The spectral characteristics of the CO_2 and climate records and the above discussion of climatic feedbacks lead to the idea that climatic changes would be triggered by insolation changes with the relatively weak orbital forcing being strongly amplified by possibly orbitally induced CO_2 changes. This is in the general line of the ideas developed by Pisias and Shackleton[27] in their modelling of the global climatic response to orbital forcing and CO_2 changes. Our interpretation must remain cautious as it does not shed light on the physical mechanisms involved; our results do not even allow us to exclude the possibility that CO_2 changes are just a consequence of climatic changes. However, the Vostok series appear to provide the most direct support so far of such an interaction between CO_2, orbital forcing and climate because (1) we have straightforward access to the climatic variables of interest (namely atmospheric CO_2 content and atmospheric temperature) and (2) a thorough examination of the climate model results does not contradict such a CO_2-amplifying role. Note also that such a climatic role for CO_2 would certainly make it easier to explain the synchronism of major glaciation in the Northern and Southern Hemispheres[10-12], one of the points left unanswered in the Milankovitch theory of the ice ages.

One can speculate from the Vostok results that the 100-kyr glacial-interglacial oscillation has its origin in the large associated CO_2 variations rather than in postulated nonlinearities associated with ice-sheet growth and decay[8,9,13-17]. Indeed, a study of the non-glacial past clearly shows that the presence of ice sheets is not a necessary condition for the existence of the ~100-kyr cycle and that other interpretations are possible[53-55].

Finally, we remain cautious about extrapolating from this analysis of past conditions to the impact of the recent anthropogenic increase in CO_2 on future climate. Even if our suggested interpretation is correct, one must realize that long-term feedbacks operate differently when going towards the last or the present interglacial than when going towards a possible future CO_2 superinterglacial. Such a prediction needs a better understanding of the mechanisms involved in this interaction between CO_2 orbital forcing and climate. We follow Broecker *et al.*[18] in calling for the development and testing of joint ocean-atmosphere models as a tool to attack this complex but extremely important problem.

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The polarization spectrum of supernova 1987A interpreted in terms of shape asymmetry

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Polarimetry done by Schwarz and Mundt[1] on the type II supernova 1987A on 6 and 7 March 1987 showed variation in polarization across line profiles (see Table 1). This polarization structure is interpreted as arising from an asymmetric, homologously expanding, scattering atmosphere surrounding an asymmetric continuum-producing photosphere. Resonant scattering of radiation by ions in the atmosphere produces the line structure in the flux spectrum and polarizes the emergent radiation. The asymmetric shape of the atmosphere causes a non-zero net polarization. Sobolev-method radiative transfer calculations with axisymmetric oblate ellipsoidal models have been carried out to fit the observed data. The models are parameterized by the ratio of the symmetry axis to the perpendicular axis, *c/a*. The fits to the 1987A data indicate that (*c/a*) is ~0.6–0.8.

The Sobolev method[2–4] is used for calculating resonant line spectra in atmospheres with large velocity gradients and has been successfully applied to supernovae by Branch *et al.*[5–7]. The usual Sobolev method relies on complete redistribution of photons in frequency and angle. Complete redistribution (CRD) in frequency means that the incoming and outgoing photon frequencies are uncorrelated and are described by independent absorption and emission profiles. CRD in angle means that the scattering is isotropic and non-polarizing. To include polarization a hybrid redistribution was considered. This hybrid redistribution has complete redistribution in frequency, but to give the angular redistribution of the scattered radiation it includes a linear combination of the isotropic and Rayleigh phase matrices (see Chandrasekhar[8] for the prescription of the matrix coefficients). The radiation fields are described in terms of the Stokes parameters. The following expressions were obtained for the Stokes parameter source functions assuming axial symmetry and no circular polarization:

$$S_l(\mathbf{r}) = S_1 + S_2\mu^2 + S_3\mu\sqrt{(1-\mu^2)}\cos\phi + S_4\mu^2\cos 2\phi \qquad (1a)$$

$$S_r(\mathbf{r}) = S_5 - S_4\cos 2\phi \qquad (1b)$$

$$S_u(\mathbf{r}) = S_3\sqrt{1-\mu^2}\sin\phi + 2S_4\sin 2\phi \qquad (1c)$$

where l indicates the radiation vector aligned with the axis of symmetry, r the vector aligned perpendicular to the axis of symmetry, and u the difference between the l and r components in a 45° rotated system. The coefficients labelled 1–5 are obtained by numerical integration over the incoming specific intensity, and by some linear algebra.

The models examined with this modified Sobolev method consist of an axially symmetric ellipsoid photosphere which produces an unpolarized, frequency-independent or Planck continuum, and a similar ellipsoid atmosphere in which there is only resonant scattering by ions. The Sobolev optical depths

Table 1 Polarimetric data for SN1987A

Feature	λ (Å)†	$Q(\%)$	$U(\%)$	$\chi(°)$	$P(\%)$
ISP‡		+0.88 ± 0.07	+0.41 ± 0.07	12.5 ± 2.7	0.97 ± 0.09
GFP		+0.24 ± 0.07	+0.18 ± 0.07	18.4 ± 9.3	0.30 ± 0.10
UBVRI (25 February 1987)§		−0.02 ± 0.07	−0.14 ± 0.07	131 ± 16	0.14 ± 0.08
UBVRI (28 February 1987)		−0.10 ± 0.07	−0.04 ± 0.07	101 ± 24	0.11 ± 0.09
UBVRI (2 March 1987)		−0.19 ± 0.07	−0.09 ± 0.07	103 ± 13	0.21 ± 0.09
UBVRI (7 March 1987)		−0.36 ± 0.07	−0.09 ± 0.07	97 ± 7	0.37 ± 0.08
Hα (peak)	6,565|10	−0.12 ± 0.07	−0.08 ± 0.07	107 ± 19	0.14 ± 0.10
Hα (trough)	6,251|33	−0.36 ± 0.07	−0.24 ± 0.07	107 ± 6	0.43 ± 0.10
Hβ (peak)	4,867|34	−0.32 ± 0.07	−0.21 ± 0.07	107 ± 7	0.38 ± 0.10
Hβ (trough)	4,697|10	−0.94 ± 0.07	−0.69 ± 0.07	108 ± 2	1.17 ± 0.10
Hγ (peak)	4,340|28	−0.30 ± 0.07	−0.02 ± 0.07	92 ± 7	0.30 ± 0.07
Hγ (trough)	4,188|33	−0.46 ± 0.07	−0.41 ± 0.07	111 ± 5	0.62 ± 0.10
Na D (peak)	5,897|56	−0.26 ± 0.07	−0.07 ± 0.07	98 ± 9	0.27 ± 0.09
Na D (trough)	5,757|20	−0.60 ± 0.07	−0.44 ± 0.07	108 ± 4	0.74 ± 0.10

Unless otherwise indicated the data were taken on the nights of 6 and 7 March 1987 (ref. 1).

† These values describe the filters used by Schwarz and Mundt[1]: for example, central wavelength|full width half maximum.

‡ ISP is the interstellar polarization and GFP is the galactic foreground polarization; ISP value from Barrett[10]; the other features have been corrected by these values to give intrinsic polarizations.

§ UBVRI labels the mean polarization of the broadband colours U, B, V, R and I.

Table 2 Spectroscopic line data for SN1987A taken on 6 March 1987

Line	λ_{peak} (Å)	F_{peak}*	λ_{trough} (Å)	F_{trough}	$\Delta\lambda$ (Å)	V_{trough} (cm s^{-1} × 10^9)	$\gamma_\mu f$†	τ_{ph}
Hα	6,562	1.95 ± 0.10	6,284	0.25 ± 0.10	278	1.27	0.4205	1,000
Hβ	4,861	1.10 ± 0.10	4,697	0.20 ± 0.10	170	1.05	0.05799	138
Hγ	4,340	0.55 ± 0.10	4,188	0.20 ± 0.10	152	1.05	0.01938	46
Na D	5,889	1.00 ± 0.10	5,757	0.60 ± 0.10	140	0.712	0.577	3.1

Data from ref. 9.

* The flux *F* is normalized to the continuum value at 5,900 Å.

† The Sobolev optical depths for a supernova, neglecting stimulated emission, are given by $\tau = 0.23\lambda_\mu f N t_d$, where λ_μ is the wavelength in micrometres, *f* is the oscillator strength, *N* is the density of absorbers in the lower level of the transition per cubic centimetre and t_d is the time in days since the explosion. Thus the optical depths for the Balmer lines always have the same ratio in this approximation.

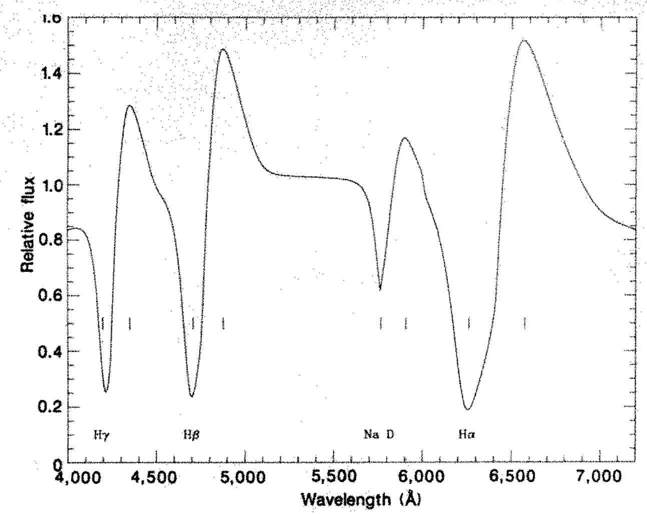

Fig. 1 A spherically symmetric model synthetic flux spectrum for 1987A on 6 March 1987 showing Hα, Hβ, Hγ and Na D lines. The fitted τ_{ph} values are 1,000, 138, 46 and 3.1 respectively. The line-of-sight velocity of that part of the photosphere nearest the observer is set to 0.71×10^9 cm s^{-1}. This value was obtained by fitting the wavelength shift of the weak Na D line's absorption trough[9]. The background continuum is a Planck spectrum with $T=5{,}500$ K. This value was obtained by interpolating 1 March and 11 March Planck spectrum fits[15]. The flux is normalized to its continuum value at 5,900 Å. The fitted τ_{ph} values from this model are used for the asymmetric models because the flux profiles are not changed drastically by varying (c/a) for $(c/a)\geq0.5$.

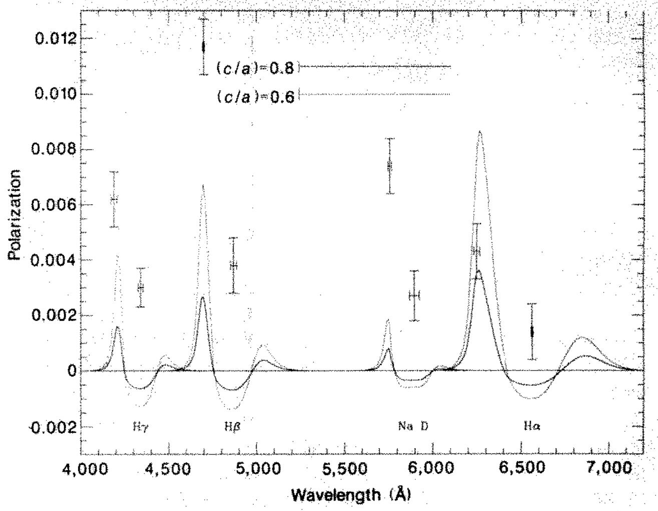

Fig. 2 Synthetic line polarization spectra for supernova 1987A (6 and 7 March 1987) for oblate ellipsoid models. The inclination angle of the symmetry axis was set to 90° (see Fig. 1 legend for a description of the other model parameters). Only the $(c/a)=0.8$ and $(c/a)=0.6$ model spectra are shown for clarity. The Schwarz and Mundt[1] data points are shown without any correction for a continuum polarization. Vertical error bars, measurement uncertainty; horizontal error bars, half-width of the filters.

were parameterized according to

$$\tau=\tau_{ph}\left(\frac{g_{ph}}{g}\right)^{p} \tag{4}$$

τ_{ph}, a fitting parameter, is the optical depth at the photosphere, and p, in correspondence to the choice of Branch *et al.* for spherically symmetric models[5-7], is set to 7. The factor g is a generalized radius parameter given by

$$g=\sqrt{(x/a)^2+(y/a)^2+(z/c)^2} \tag{5}$$

where c and a are, respectively, the semiaxes parallel and perpendicular to the axis of symmetry.

A parameter survey of asymmetric models allows several general conclusions to be drawn that apply to 1987A. (1) There is polarization structure associated with the P-Cygni line profiles. (2) There is in most cases an inversion in the polarization profile (a 90° shift in polarization angle) between the P-Cygni peak and trough features for both oblate and prolate models. The peak polarization vector aligns parallel to the long axis of the model and the trough polarization vector is perpendicular to this axis. (3) Prolate and oblate models for the same degree of asymmetry, and with $2\geq(c/a)\geq0.5$, have similar degrees of polarization. (4) The magnitude of trough polarization is typically more than twice that of peak polarization. (5) The trough feature is typically narrower than the peak feature. (6) The polarization values obtained are not very large even for large asymmetries. For example, for a typical prolate ellipsoid model the difference between peak and trough polarization is ~1.7% when $(c/a)=2$ and with other parameters chosen to maximize polarization. For a typical oblate ellipsoid model with $(c/a)=0.5$ the difference between peak and trough polarization is also ~1.7%.

There is spectropolarimetric data for four of 1987A's lines[1]: Hα, *Hβ*, Hγ and Na D (see Tables 1 and 2). None of the lines was a pure, unblended resonant scattering line on 6 March[9]. The Hα line shows more emission than absorption, while the reverse is true of a resonant scattering line. There may be a thermal source of photons and this would tend to depolarize this line's flux. The Hβ, Hγ and Na D lines are not unblended although the absorption features of the Hβ and Na D line are well defined. A synthetic spectrum for these lines for a spherically symmetric model is given in Fig. 1. The fitted τ_{ph} values from this model have been used for the asymmetric model calculations because the shapes of the flux profiles do not change drastically for values of $(c/a)\geq0.5$. The inclination angle of the symmetry axis of the asymmetric models was set to 90° because the large polarization of the Hβ line could not be fitted by substantially smaller angles. Figure 2 shows polarization profiles for oblate models with varied (c/a). Oblate models were studied because oblateness has an obvious physical cause in the rotation of a supernova progenitor.

The polarimetric data[1] (corrected for interstellar polarization by using Barrett's value[10]) do not exhibit the polarization inversion predicted by the Sobolev models (see Table 1). It may be

Table 3 Observational and model ΔP values

Line	ΔP_{obs} (%)	ΔP_{model} (%)* $(c/a)=1$	$(c/a)=0.8$	$(c/a)=0.6$	$(c/a)=0.5$	$(c/a)=0.25$
H_α	0.29 ± 0.20	0.00	0.40 ± 0.03	0.90 ± 0.03	1.17 ± 0.03	1.61 ± 0.03
H_β	0.79 ± 0.20	0.00	0.33 ± 0.02	0.79 ± 0.02	1.09 ± 0.02	2.00 ± 0.02
H_γ	0.32 ± 0.17	0.00	0.16 ± 0.04	0.36 ± 0.04	0.46 ± 0.04	0.56 ± 0.04
Na D	0.47 ± 0.19	0.00	0.10 ± 0.03	0.21 ± 0.03	0.26 ± 0.03	0.32 ± 0.03

* The uncertainties assigned to the ΔP_{model} values are only the numerical uncertainties.

that there is a continuum polarization upon which the line polarization profiles are superimposed. Assuming this to be the case the peak-to-trough polarization difference ΔP becomes the quantity to be fitted. The ΔP values for the observational and model lines are given in Table 3. Recalling that none of the lines is a pure resonant scattering line, it is not surprising that the best fitting (c/a) values are different for each line. But the Balmer lines are fitted by (c/a) values in the range 0.6–0.8 and the conclusion is drawn that supernova 1987a is 20 to 40% oblate asymmetric.

Shapiro and Sutherland[11] considered continuum polarization from ellipsoidal supernovae and McCall[12], line polarization. Their physical models are somewhat different from that of this letter. But from their results, estimates of 1987A's asymmetry can be made that are also ~20–40%.

Müller and Hillebrandt's[13] rotating core explosion models show core oblateness of $\gtrsim 20\%$ for core rotational energies $\gtrsim 10^{49}$ erg. Rotational energies of a few 10^{49} erg are attributed to pulsars[14], the presumed remnants of type-II supernovae. The core oblateness may be communicated to the atmosphere, where it would become the source of the polarization. The details of Sobolev polarization calculations and further fits will appear in a paper now in preparation.

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Modelling of the radio burst from SN1987A

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Observations at Australian observatories[1] showed that radio emission from SN1987A in the Large Magellanic Cloud[2] reached a peak of ~100 mJy at frequencies of ~1 GHz within three days of the optical rise and then declined on a similar timescale. This behaviour is different from that observed from previous extragalactic type II radio supernovae[3] where the peak luminosity is two to three orders of magnitude greater and the emission rises tens or even hundreds of days after the supernova event and then decays with a timescale of years. Here we propose that the radio emission mechanism for SN1987A is the same as that proposed for previous type II supernova[4], namely, synchrotron emission from a thin shell near the edge of the expanding ejecta. The differences are mainly due to the absence of a thick circumstellar envelope around the progenitor star, Sk −69 202, at least in the line of sight to the supernova. We discuss several possible models for the emission and absorption processes and set limits on the density of a circumstellar envelope.

We assume that the relativistic electrons were located in a shell of radius R and thickness D located at the outer edge of the expanding ejecta. The shell is assumed to expand with $R \propto t^m$ where t is the time since the supernova explosion and m is a constant <1 allowing for deceleration of the shell expansion (see ref. 4). The relativistic electrons could have been accelerated during the explosion by, for example, first-order Fermi acceleration as the supernova shock propagated through the stellar atmosphere (see ref. 5). Magnetic fields would have been trapped in the expanding ejecta and on day 2 (25 February) field strengths in the shell of the order of a milligauss are reasonable. For synchrotron radiation in the radio band, electron Lorentz factors $\gamma_e \sim 100$ are implied and the gyro-radius is much less than the thickness of the shell, so the relativistic electrons would be contained by the magnetic field in the shell. The relativistic electrons are assumed to have a power-law differential energy distribution

$$N(E)\,\mathrm{d}E = KE^{-\gamma}\,\mathrm{d}E \tag{1}$$

The synchrotron radiation then has an emissivity of

$$j_\nu \propto a(\gamma) K B^{(\gamma+1)/2} \nu^{-(\gamma-1)/2} \tag{2}$$

where $a(\gamma)$ is a slow function of γ, B is the magnetic flux density and ν is the radio frequency[6]. To produce the observed radio luminosity of $\sim 2\times 10^{33}$ erg s^{-1}, the total energy in the synchrotron electrons is $\sim 10^{44}$ erg, a minute fraction of the total energy output of the supernova.

The relativistic electrons will suffer energy losses due to a number of processes. Synchrotron losses are unimportant having a timescale of thousands of years. Adiabatic losses have a timescale of RV^{-1}, where V is the expansion velocity, and hence are significant on the timescale of the event. In the intense radiation field of the supernova, inverse Compton losses are likely to be important. The timescale for this process τ_{IC} scales as $R^2(\gamma_e L_r)^{-1}$, where L_r is the radiative luminosity of the supernova. Initially L_r was dominated by optical and ultraviolet emission and was $\sim 10^{41}$ erg s^{-1}. For $R \sim 10^{14}$ cm (appropriate for day 2) and $\gamma_e \sim 100$, $\tau_{\mathrm{IC}} \sim 3\times 10^5$ s. Therefore, at times of interest, adiabatic losses dominate. It is possible that the energy losses due to these processes continued to be overcome by generation of relativistic electrons (see ref. 4) and we consider models where this is and is not the case.

Absorption processes which may be important include free-free absorption and synchrotron self-absorption. The successful interpretation of previous extragalactic type II supernovae[3,4] is based on free-free absorption in a circumstellar envelope with an r^{-2} density profile, presumably generated during a red giant phase of the progenitor star. For SN1987A any circumstellar envelope must be relatively thin, at least in the line of sight to the supernova, otherwise the prompt radio burst would not have been observed. The progenitor star for SN1987A, Sk −69 202, was apparently a B3 supergiant star[7] which would not be expected to have an extensive circumstellar envelope, and indeed, there is no evidence for one in either the optical or ultraviolet spectra[8,9]. The coefficient of free-free absorption can be approximated by

$$k_{\mathrm{ff}} = 0.108\, T_e^{-3/2} n_{\mathrm{th}}^2\, \nu^{-2}\ \mathrm{cm}^{-1} \tag{3}$$

where T_e is the electron temperature and n_{th} is the thermal electron density[10]. For T_e of a few thousand degrees, a limit on $n_{\mathrm{th}} \lesssim 10^6$ cm^{-3} in or near the emission region on day 2 is implied by the requirement that the free-free optical depth be $\lesssim 1$ at frequencies of ~1 GHz. The emission region must therefore be close to the outer edge of the expanding ejecta and any circumstellar envelope must be thin.

If thermal plasma exists in the emission region, Razin-Tsytovich suppression[10] of the synchrotron emission may be important. The criterion for this not to be the case is that $\nu > \gamma_e \nu_p$ where ν_p is the plasma frequency. For day 2, this implies a limit on n_{th} similar to that obtained for free-free absorption. Therefore we do not expect this effect to be important at the times of interest. Finally, synchrotron self-absorption may be significant.

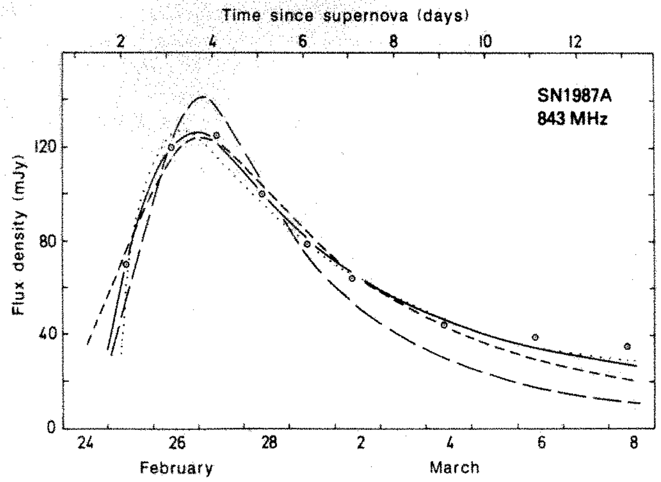

Fig. 1 Observed time dependence of the 843-MHz flux density of SN1987A from Turtle *et al.*[1] and the fits of four different models to these data. Observational errors are ±2 mJy. For models 1 and 2, both thermal and relativistic electrons are contained in a relatively thin expanding shell of constant thickness located near the edge of the ejecta. Free-free absorption and synchrotron self-absorption are included in the fits. For model 1, the coefficient K is assumed to vary as R^{-2}, whereas in model 2 adiabatic expansion of the relativistic electrons is assumed. Model 3 is similar to model 2 except that the emitting and absorbing shell is assumed to expand with $D \propto R$. For model 4, free-free absorption occurs in a r^{-2} circumstellar envelope and all other absorption processes are neglected. The assumptions for the synchrotron-emitting shell are as in model 1. ——, Model 1; - - - -, model 2; — —, model 3; · · · · ·, model 4.

The absorption coefficient for this process is given by[6]

$$k_{\mathrm{syn}} \propto g(\gamma) K B^{(\gamma+2)/2} \nu^{-(\gamma+4)/2} \tag{4}$$

where $g(\gamma)$ is a slowly varying function of γ.

We consider four models for the prompt radio burst. For each model, we have made a least-squares fit to the 843-MHz data from the Molonglo Observatory[1]. These data have the highest sensitivity of those available and show both the rise and the fall of the emission most clearly. Time-dependent parameters for the models are quoted at time t_0, 25.4 February UT or 2.1 days after the supernova explosion, the time of the first Molonglo observation. For all fits, the deceleration parameter $m = 0.99$, corresponding to essentially free expansion.

In models 1 and 2, thermal electrons are assumed to coexist with the synchrotron electrons in an expanding shell of constant thickness $D = \zeta R_0$ where $R_0 = R$ at t_0. Any external wind envelope is assumed to be of negligible density. The magnetic field is assumed to be random and hence $B \propto R^{-1}$. It is further assumed that, as the shock went through the outer layers of the star, the shocked gas was able to cool and that subsequently the expansion was adiabatic and hence $T_e \propto R^{-4/3}$ for the times of interest. At t_0 a value of 5,000 K is assumed. The optical depth for free-free absorption along a radial path is then

$$\tau_{\mathrm{ff}} = k_{\mathrm{ff}} D \propto \zeta R^{-2} \nu^{-2} \tag{5}$$

where the constant of proportionality is determined by R, n_{th} and T_e at the reference time t_0.

For model 1, the coefficient of the relativistic electron energy distribution, K, is assumed to vary as R^{-2}. This requires that acceleration processes continue to operate as the shell expands and have a short timescale compared to the dissipative processes (see ref. 4). The index γ is assumed to remain constant during the expansion. The optical depth for synchrotron self-absorption is then

$$\tau_{\mathrm{syn}} = k_{\mathrm{syn}} D \propto \zeta g(\gamma) K_0 B_0^{(\gamma+2)/2} R^{-(\gamma+6)/2} \nu^{-(\gamma+4)/2} \tag{6}$$

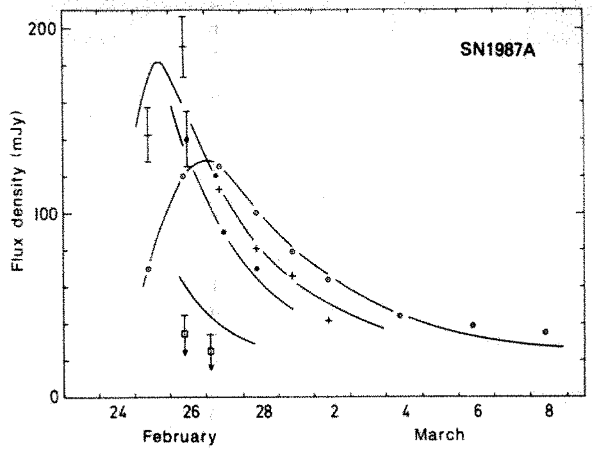

Fig. 2 Predicted flux densities from model 1 compared to the observations at all four radio frequencies. The apparent time of the supernova event[12,13] was 23.3 February. Observations at 843 MHz (⊙), 2.3 GHz (●) and 8.4 GHz (⊡) are from Turtle *et al.*[1]. Flux densities at 1.4 GHz (+) from the Fleurs observations have been revised upward by ~35% (J. D. Bunton, personal communication). Representative error bars are given for some of the points.

where K_0 and B_0 are the values of K and B at t_0, respectively. From the equation of radiative transfer, the observed flux density is

$$S = \pi (R/d)^2 (j_\nu / k_\nu)(1 - e^{-\tau}) \tag{7}$$

(neglecting geometric effects), where d is the distance to the supernova, assumed to be 55 kpc, $k_\nu = k_{\mathrm{syn}} + k_{\mathrm{ff}}$ and $\tau = \tau_{\mathrm{syn}} + \tau_{\mathrm{ff}}$. The observed flux density rises with time until $\tau \sim 1$ due to the dominant effect of the decrease in absorption and then falls due to the decreasing synchrotron emissivity as the emitting shell expands.

For model 2, we assume that there is no further acceleration of the relativistic electrons and that their expansion is adiabatic at the times of interest. For this case γ remains constant during the expansion and $K \propto R^{-(2\gamma+4)/3}$. Then $j_\nu \propto R^{-(7\gamma+11)/6}$ and $k_{\mathrm{syn}} \propto R^{-7(\gamma+2)/6}$.

For an essentially free expansion, one might expect the synchrotron-emitting shell to expand with thickness proportional to radius. Model 3 is the same as model 2 except that $D \propto R$. For this case, $K \propto R^{-(\gamma+2)}$, $B \propto R^{-2}$, $n_{\mathrm{th}} \propto R^{-3}$ and $T_e \propto R^{-2}$. Hence $j_\nu \propto R^{-(2\gamma+3)}$, $\tau_{\mathrm{ff}} \propto R^{-2}$ and $\tau_{\mathrm{syn}} \propto R^{-(2\gamma+3)}$.

Finally, we take the model of Chevalier[4] in which free-free absorption occurs in a circumstellar wind with $n_e \propto r^{-2}$. We assume $T_e = 5{,}000$ K in the wind. Absorption due to other processes is assumed negligible and K is taken to be $\propto R^{-2}$ as in model 1. As the shock expands the free-free optical depth in the line of sight from the shock front decreases as R^{-3}.

Figure 1 shows the fits of these models to the Molonglo data and the derived parameters are given in Table 1. In all four models the rapid rise and more gradual fall of the observed emission is reproduced. Model 1 gives the best fit with only the last two points being significantly off the fitted curve. Optical depths are small by day 4, so after this time the spectral index will be $\alpha = -(\gamma - 1)/2 \sim -0.7$ in accordance with the observations[1]. Model 2, where the relativistic electrons are assumed to expand adiabatically, also gives an acceptable fit with the thickness of the emitting shell being about three times larger than for model 1. For model 3 it was necessary to hold ζ constant to obtain convergence of the least-squares procedure. This model, where $D \propto R$, is clearly inferior with both the rise and fall of the emission being too steep. This more rapid evolution results from the stronger R-dependence of the various parameters. Both the relativistic and thermal electron densities are much higher in this model and the absorption optical depths

Table 1 Parameters from least-squares fits to Molonglo data

	V_0 (km s^{-1})	ζ	K_0 (c.g.s. units)	γ	B_0 (gauss)	$n_{\text{th}0}$ (10^5 cm^{-3})
Model 1	18,600	0.048	8.7	2.33	0.0028	5.32
Model 2	22,100	0.173	16.2	2.06	0.0045	4.52
Model 3	21,200	0.200*	46.1	2.23	0.0138	16.80
Model 4	21,900	0.249	24.9	1.79	0.0056	1.73

* Fixed parameter.

are large for a long time and so this model does not give a good fit to the observed spectrum. For model 4, the external r^{-2} wind case, we had to set arbitrarily the synchrotron self-absorption to zero, as the least-squares fit gave a negligible density for the external wind if this was not done. This indicates that the model is not a good fit to the data as can be seen from Fig. 1. The exponential rise is too steep and it is not possible to fit the first three data points within their errors. Because the model 4 assumptions about the emission zone are similar to those for model 1, this model gives a good fit at later times when the free-free optical depth is small.

In Fig. 2 we show the flux densities predicted at the other observed frequencies using model 1 and the corresponding parameters given in Table 1. Overall, the model gives a good prediction of the observed flux densities (given that the error bars at the other frequencies are much larger than those at 843 MHz), especially at late times when absorption processes are negligible. The main discrepancies are that the predicted peak at 1.4 GHz occurs ~12 h too early and that the predicted flux densities at 8.4 GHz are ~80% too high. The observed later peak at 1.4 GHz implies a larger effective absorption coefficient at higher frequencies, at least initially, perhaps as a result of an initial relaxation of the density profiles in the emitting region. The lower flux densities at 8.4 GHz imply a steepening of the relativistic electron energy distribution at high energies which may result directly from the acceleration mechanism, but probably reflects the effects of energy-loss processes such as inverse Compton scattering. In addition, in all models, the derived expansion velocity $V_0 \sim 20{,}000$ km s^{-1} is less than the maximum blueshift observed in optical[8] and ultraviolet[9] spectra. Further detailed modelling including more realistic density profiles for the ionized and relativistic gas is required to assess the significance of these discrepancies. The derived magnetic flux densities of a few milligauss at t_0 imply a stellar field of the order of a gauss, not inconsistent with that expected for a B3 star[11]. The overall agreement of the model with the observations illustrated in Fig. 2 indicates that the basic model, synchrotron emission from an expanding shell together with free-free absorption from plasma in the emission region and/or synchrotron self-absorption, is correct.

Perhaps the most significant result from this study is that the density of thermal electrons, either in the emission region or in the line of sight to the supernova, is small. In particular, the value of $n_{\text{th}0}$ quoted in Table 1 for model 4, the circumstellar wind model, is an upper limit on the actual density because other absorption processes have been neglected. If we assume the wind is fully ionized, we can take this as a limit on the gas density. The implied limit on mass-loss rate for the progenitor star is proportional to the assumed wind velocity; for 100 km s^{-1} it is 10^{-7} $M_\odot$ yr^{-1}, much smaller than the mass-loss rate expected from a red giant. This indicates either that the progenitor star did not go through a red giant phase or, if it did, that the mass outflow was nonspherical and such that our line of sight does not intercept the bulk of the circumstellar material.

This apparent lack of an extensive circumstellar envelope also accounts for the low luminosity of the prompt radio burst from SN1987A. In type II supernovae where there is a massive circumstellar envelope, the synchrotron electrons are probably generated by turbulent interactions as the shock encounters the envelope. For SN1987A the low circumstellar density may have resulted in a very different shock. If a massive but non-spherical circumstellar envelope exists, the shock may still encounter this, perhaps resulting in a radio burst similar to those observed in previous type II SN. As mentioned by Turtle *et al.*[1], the prompt burst observed for SN1987A could not have been detected for any previous event.

There may be other, more centrally located, sources of radio radiating electrons. Relativistic electrons generated deeper within the ejecta by the initial passage of the shock and still behind absorbing layers lose energy through Coulomb and Compton collisions and no longer radiate at radio frequencies. However, a central energy source such as a pulsar may continue to generate relativistic electrons and the synchrotron radiation from such electrons may become observable at a later date. If there is no such central energy source and no massive circumstellar envelope it is possible that there will be no significant radio emission until (portions of) the ejecta with sufficient energy reach regions of the interstellar medium which are sufficiently dense for turbulent acceleration of electrons to occur. If the supernova is surrounded by a large cavity, this may never happen. We then have the interesting possibility of a supernova which apparently formed a neutron star[12,13] having no associated radio remnant.

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Oxygen stoichiometry, superconductivity and normal-state properties of $YBa_2Cu_3O_{7-\delta}$

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Probably the most unusual crystal-chemical aspect of the new high-temperature oxide superconductors is their significant variability in oxygen content. For the $YBa_2Cu_3O_{7-\delta}$ family of compounds, the oxygen stoichiometry varies in the range $0 < \delta < 1$ under normal synthetic conditions, with the optimal 91-K superconductive transitions obtained near $\delta = 0$ (see, for example, ref. 1). Work presented so far on the dependence of both superconducting and normal-state properties on oxygen stoichiometry has considered only the value of δ in $YBa_2Cu_3O_{7-\delta}$. Here we present data that indicate the issue to be considerably more interesting and complex, and propose that the microscopic variables thus far not considered include the distribution of oxygen over the available sets of sites in the structure, with that distribution strongly dependent on synthetic conditions. In addition to the dependence of the bulk transition temperture (T_c), normal-state resistivity and crys-

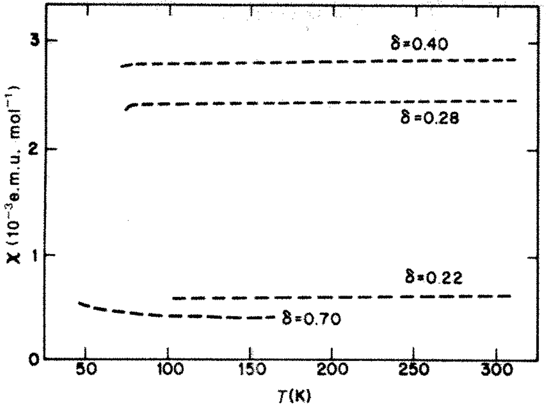

Fig. 1 Temperature dependence of magnetic susceptibility for $YBa_2Cu_3O_{7-\delta}$ samples prepared by the gettered annealing technique.

tallographic symmetry on the microscopic distribution, we find the magnitude of the magnetic susceptibility in the normal state to be strongly affected by the microscopic state of the oxygen subsystem. We also report the existence of a single-phase 60-K bulk superconductor at stoichiometry $YBa_2Cu_3O_{6.6-6.7}$, which we postulate to have an ordered array of oxygen vacancies, and two distinct plateau regions in the variation of T_c with x which are either unobserved or only hinted at under other synthetic conditions.

The oxygen-deficient samples were prepared from single-phase, polycrystalline $YBa_2Cu_3O_7$ by a low-temperture gettered annealing technique[2]. Pellets were sealed in evacuated quartz tubes with bright zirconium foil, annealed at temperatures between 360 and 520 °C for two days and air-quenched. For the sample sizes used, the amount of oxygen gettered from the $YBa_2Cu_3O_7$ is dependent on both the temperature of the anneal and the surface area of the zirconium foil. The technique results in extremely uniform oxygen-deficient samples and allows ample time for ordering of oxygen defects, if any is to occur. All samples were based on the same master batch of single-phase $YBa_2Cu_3O_7$ powder. Pellets were sintered at 1,000 °C in oxygen for 16 h, then annealed at 500 °C in oxygen for 16 h, and furnace-cooled to room temperature. Oxygen content after gettered annealing was determined by weight loss on heating in hydrogen to 1,000 °C. Other parts of the samples were used for powder X-ray diffraction, d.c. magnetization (SQUID magnetometer) and resistivity (four-probe a.c., 26 Hz) measurements.

Figure 1 shows the temperature dependence of the magnetic susceptibility (χ) in the normal state for selected oxygen-deficient samples prepared by gettered annealing. χ is either independent of temperature or decreasing with temperature for all δ up to 0.5, with only the slightest sign of a moment appearing at $\delta = 0.70$. This is in considerable contrast to our earlier published results on a $\delta = 0.1$ sample[3], which showed a local moment of 0.9 Bohr magnetons (μ_B) per formula unit, and the Curie-Weiss behaviour of χ observed by many groups (see for instance refs 5-7). The local moment (ref. 4 and H. Katayama-Yoshida, personal communication) can either be intrinsic, or due to the presence of impurities in the samples. As our materials are phase-pure (by X-ray diffraction) at better than the 1% level, we believe that the presence or absence of the local moment is an intrinsic effect worthy of further study. The synthetic conditions described here for $YBa_2Cu_3O_7$ result reproducibly in material without a local moment.

For materials prepared by oxygen removal from $YBa_2Cu_3O_7$ by flowing gas[5-7] at elevated temperatures, $\chi(T)$ is Curie-Weiss-like, with increasing local moment as a function of increasing oxygen deficiency or a δ-independent moment of 0.9 μ_B f.u.$^{-1}$ for $0 < \delta < 0.5$ which then increases to 1.9 μ_B f.u.$^{-1}$ at $\delta = 0.9$. As shown in Fig. 1, material can be prepared by the zirconium gettering technique at a stoichiometry of $YBa_2Cu_3O_{6.3}$ without significant local moments.

Table 1 Orthorhombic cell parameters and resistivities of $YBa_2Cu_3O_x$ at room temperature

x	a (Å)	b (Å)	c (Å)	ρ (mΩ cm)
7.0	3.822 (1)	3.891 (1)	11.677 (2)	1-1.5
6.78	3.827 (2)	3.895 (2)	11.722 (7)	5
6.76	3.830 (2)	3.898 (2)	11.728 (6)	4.9
6.72	3.835 (4)	3.890 (4)	11.716 (11)	3.8
6.60	3.831 (2)	3.889 (2)	11.736 (6)	3.5
6.53	3.838 (2)	3.887 (2)	11.747 (6)	4.7
6.51	3.845 (3)	3.887 (3)	11.768 (8)	5.4
6.30	3.851 (5)	3.883 (5)	11.789 (16)	9.2

Comparison of published data and those presented here reveals that specification of the total oxygen stoichiometry in $YBa_2Cu_3O_{7-\delta}$ is not sufficient to characterize the oxygen subsystem. We propose that the magnetic susceptibility is a sensitive measure of the local copper environment, especially with regard to the microscopic oxygen configuration. The variables thus far 'hidden' in the problem are the relative occupancies of the sites available for the oxygen within the cation array. Referred to the well-known $YBa_2Cu_3O_7$ structure (see, for example, ref. 8), the oxygen sites we consider relevant are the $(0, \frac{1}{2}, 0)$ site in the copper-oxygen chains, which would be fully occupied in an ideal structure, the $(\frac{1}{2}, 0, 0)$ site between chains, completely vacant in the ideal structure, and the $(\frac{1}{2}, 0, \frac{3}{8}; 0, \frac{1}{2}, \frac{3}{8})$ sites in the copper-oxygen planes, fully occupied in the ideal structure. Recent neutron diffraction work[9] shows that $(0, \frac{1}{2}, 0)/(\frac{1}{2}, 0, 0)$ disorder occurs over a wide range of oxygen stoichiometry at temperatures above 450 °C. We propose that it is the relative occupancies of these sites that determine the normal-state magnetic susceptibility and resistivity, the crystallographic symmetry and unit-cell axis lengths, and the superconducting transition temperature; the configuration of the oxygen subsystem especially influences the magnetic state of the copper atoms.

The relative occupancy of the oxygen sites depends critically on the thermal history of the samples, and the final oxygen configuration of an oxygen-deficient sample may depend on the initial oxygen configuration. The $YBa_2Cu_3O_{6.3}$ sample prepared by gettered annealing from a $YBa_2Cu_3O_7$ sample with a well ordered oxygen array remains orthorhombic (see Table 1), probably because that initial ordering is at least partially maintained. $YBa_2Cu_3O_{6.3}$ prepared in the usual manner at high temperatures, where there is significant oxygen disorder, reflects that disorder, and is well into the compositional region of tetragonal symmetry.

Figure 2 shows the results of the resistive measurements of T_c for materials prepared by zirconium gettering as a function of oxygen stoichiometry. The 10-90% transition widths, indicated by the vertical bars, are quite narrow, except at the oxygen concentration where T_c is changing quickly. The narrow transition widths indicate that the oxygen in the samples is homogeneously distributed. The double plateau structure at 90 K and 60 K is dramatic and unmistakable. This plateau is either not observed, or only suggested, in materials prepared at higher temperatures by flowing gas or quenching techniques. The materials are all single-phase and orthorhombic. The amount of magnetic flux exclusion below T_c is equivalent for the 90-K and 60-K materials, indicating that the $YBa_2Cu_3O_{6.6-6.7}$ phase is also a good bulk superconductor. The resistivity of this compound shows a metallic temperature dependence. Further details of the characteristics of these materials will be reported elsewhere[10]; room-temperature resistivities are included in Table 1. Superconducting material with a bulk T_c of 55 K and a semiconducting resistivity characteristic has been prepared in the same stoichiometry range as our metallic 60-K material by a different synthetic technique[11]; in our model of the importance of microscopic oxygen configuration, dependent on thermal history, such a difference is not unexpected.

Figure 2 also shows the value of the magnetic susceptibility at 100 K for the zirconium-annealed samples as a function of

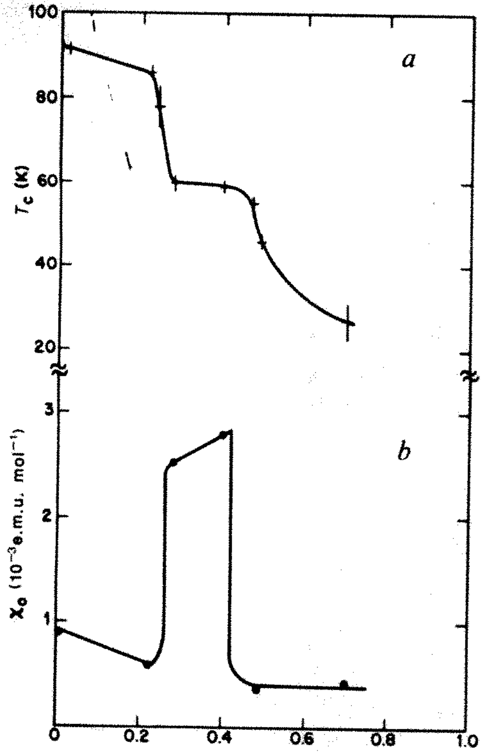

Fig. 2 *a*, Oxygen-content dependence of the resistive superconducting transition temperature in $YBa_2Cu_3O_{7-\delta}$. Vertical bars indicate the width of the transition from 90% to 10% of the normal-state resistivity, with the midpoint of the transition marked with a cross. *b*, Magnetic susceptibility at 100 K as a function of oxygen content in the same stoichiometry range.

oxygen stoichiometry. The data show a dramatic increase in susceptibility in the same composition region where the 60-K T_c plateau is observed. We do not have a detailed microscopic explanation for this non-monotonic behaviour of χ versus δ, but we suggest that in the range of δ in which χ is high, the oxygen subsystem, when annealed at the right temperature, assumes a microscopic ordering which leads to new short-range periodicities. Such order then produces new features in the energy-level scheme with a high density of states at the Fermi level.

Our results suggest that the value of the normal-state magnetic susceptibility, χ_0, is very sensitive to the microscopic oxygen configuration, and that using it as a probe of the normal electron state requires consideration of the factors discussed here. Careful preparation and subsequent characterization are needed, to be able to draw meaningful conclusions.

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Detection of oxygen ordering in superconducting cuprates

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The recent discovery of superconductivity in ceramic cuprates[1] continues to lead to spectacular increases in the critical temperature. Results from several neutron diffraction experiments have shown that the system $YBa_2Cu_3O_7$ displays long-range order in the disposition of its oxygen vacancies, giving rise to two- and one-dimensional features in the structure[2,3], which may have important electronic implications[4,5]. Although diffraction techniques have proved invaluable in determining the overall structure, the local structure should also be investigated on a microscopic level. Because of the relatively small scattering power of the light oxygen atoms, lattice images obtained from very thin films of the new superconductors are insensitive to the precise arrangement of the oxygen vacancies. It is known however, that due to dynamical effects, the thickness dependence of lattice images can be extremely sensitive to small effects, such as ionicity[6]. This can be exploited to detect the presence of long-range order in the oxygen vacancies[7]. Other investigators have produced experimental[8] or simulated[9] images, which also purport to provide evidence for the presence or detectability of order. Indications of superconductivity at very high temperatures in multiphase materials[10], and the potential importance of determining the oxygen arrangement in small regions of a multiphase sample, mean that it is important to establish in detail the conditions needed for a reliable microscopic investigation of the presence of long-range order in these materials. Here we present theoretical and experimental evidence that, under a carefully chosen and controlled set of experimental parameters, it is possible to detect the presence of long-range order in the disposition of the oxygen vacancies by high-resolution transmission electron microscopy.

We study the lattice imaging of $YBa_2Cu_3O_7$ at 400 kV and discuss the oxygen arrangement in the basal plane. Similar arguments apply to the oxygen vacancy at the Y plane and to imaging at lower accelerating voltages. We assume that no systematic changes occur in the metal-frame stoichiometry with increasing sample thickness. Our simulations[11] show that the small difference between the lengths of the *a* and *b*-axes has no significant effect on the images. Assuming complete ordering of the oxygen vacancies as shown in Fig. 1, the problem of the detection of order is equivalent to a distinction between the [100] projection, when the O-Cu-O chains lie perpendicular to the electron beam, and the [010] projection, when the chains are in the direction of the beam. Figure 2 shows the Pendellosung plots for these two projections. (SHRLI suite of multi-slice programs[11], 128 square array, acc. voltage: 400 kV, C_s: 1 mm, Δ: 100 Å, beam divergence: 1 mr, objective aperture: 1.6 Å^{-1}. The simulations do not depend sensitively on the choice of parameters.) Significant differences in the beam phaes and amplitudes only begin to appear at thicknesses ≥80 Å. This implies that a distinction between the [100] and [010] projections can only be made reliably in thicker regions of the crystal. Without information about the presence or degree of order, it is also necessary to distinguish between the ordered and disordered configurations. Figure 3 shows a series of image simulations carried out for a sample with various degrees of order in the [100] and [010] orientations. The defocus value shown is −500 Å, which produces high-contrast images easily recognizable in an experiment. Similar simulations have been made for $-300 \leq \Delta f \leq -900$ Å. These clearly show that it is difficult, if not impossible, to distinguish reliably between the ordered [100]

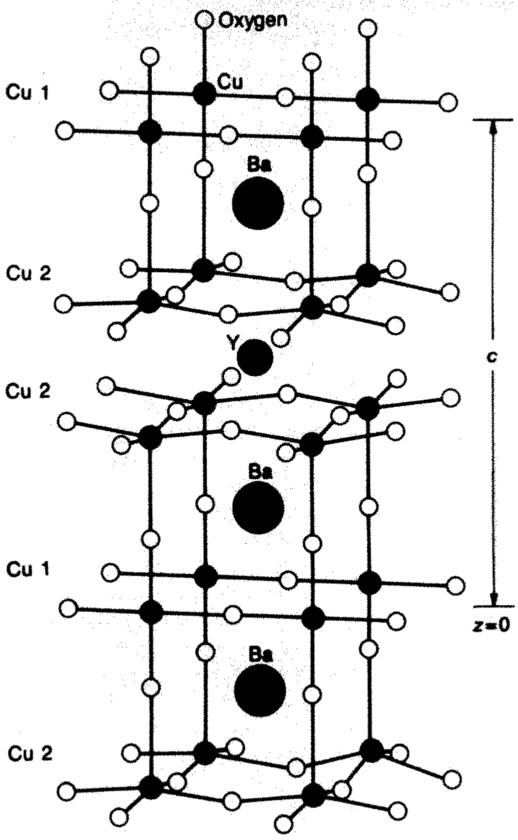

Fig. 1 Schematic representation of the structure of $YBa_2Cu_3O_7$.

and disordered configurations, essentially at any defocus or thickness. Even when the degree of order is less than one, however, the ordered [010] arrangement is distinct both from the [100], and the disordered arrangement, provided images are obtained from regions of sufficient thickness (≥ 100 Å).

In the [010] projection, when the O-Cu-O chains lie in the direction of the beam, the presence of order gives rise to a characteristic strong feature at the basal plane, which becomes a continuous white line with increasing thickness. Our simulations show that, for $-300 < \Delta f < -600$ Å and $90 < t < 135$ Å, the presence of this continuous line constitutes a unique fingerprint for the presence of O-Cu-O chains parallel to the electron beam. The key to the detection of oxygen ordering thus lies in the observation of a series of images as a function of thickness, similar to those shown in Fig. 3*c* and *d*. These are readily distinguishable from the ordered [100] and disordered configurations. We find, moreover, that oxygen desorption from the material (the uniform removal of oxygen from all basal sites, reducing the stoichiometry to $YBa_2Cu_3O_6$) also destroys the continuous white line, the fingerprint of the ordered [010] arrangement (Fig. 3*e*). The quantification of the degree of order (a distinction between rows *c* and *d*) is likely to be experimentally difficult and may be unreliable (see below).

Although the simulations of Fig. 3 show clear differences between the different oxygen configurations under ideal conditions, they place severe restrictions on the experimental conditions to be achieved before a distinction can be made. We believe that misalignments such as astigmatism can be detected with sufficient accuracy by optical diffractometry. But small tilt misalignments are difficult to detect and can seriously modify lattice images obtained from thicker crystals, and the question arises whether such misalignments render the detection of oxygen ordering impractical or unreliable. Figure 4 shows a series of simulations for the [100] and [010] projections as a function of tilt misalignment. (We assume that the major effect is due to small errors in aligning the crystal orientation with respect to

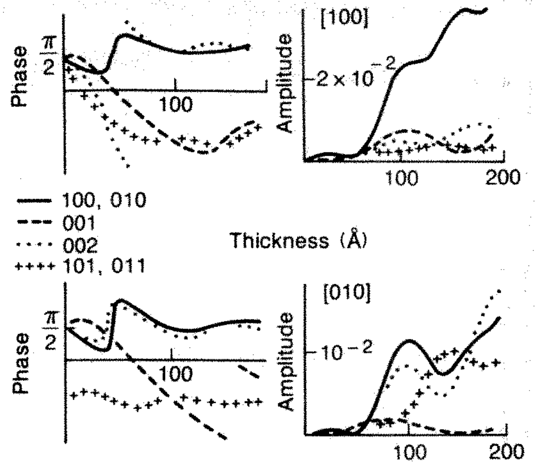

Fig. 2 Pendellosung plots showing the thickness dependence of various beams for the [100] and [010] projections in the presence of perfect oxygen order. Phases and amplitudes are referred to those of the undiffracted beam. Note that significant differences appear at thicknesses ≥ 80 Å. At such thicknesses, the behaviour of the beams depends rather sensitively on the oxygen configuration and order parameter.

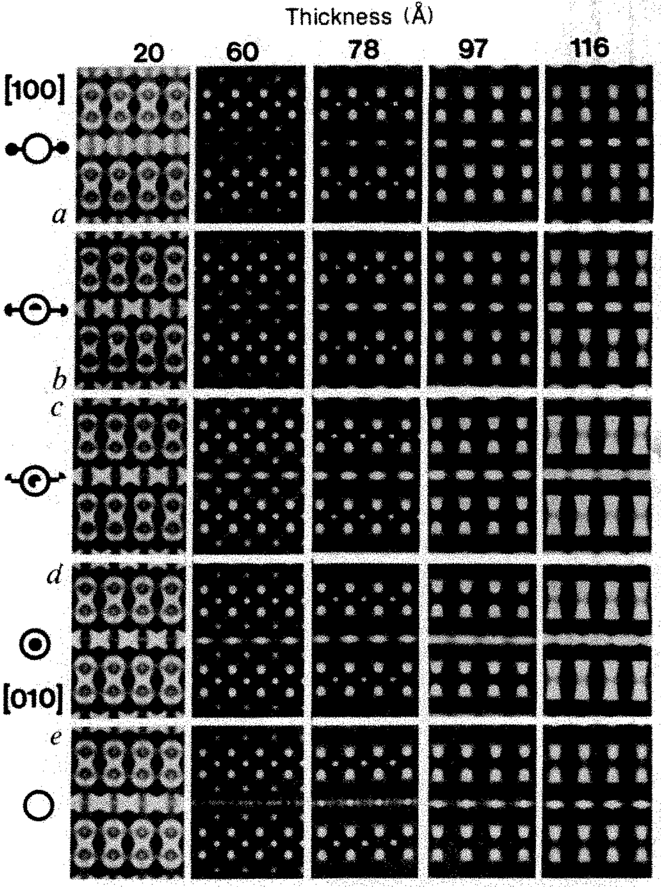

Fig. 3 Simulated lattice images at different foil thicknesses for various oxygen configurations. Unless stated, the usual orthorhombic lattice parameters are used. Defocus, −500 Å; *a*, [100] projection, perfect order; *b*, [100] (or [010]) projection in the absence of order ($a = b = 3.87$ Å); *c*, [010] projection with 75% of the oxygens lying along *b* and 25% lying along *a*, producing a degree of the order of 0.5; *d*, [010] projection, perfect order; *e*, [100] or [010] projection, $YBa_2Cu_3O_6$ ($a = b = 3.87$ Å). Note that rows *a*, *b* and *e* are similar, but that for foil thicknesses ≥ 100 Å, the continuous white line between the basal planes distinguishes rows *c* and *d*, acting as a fingerprint for the presence of order in the [010] projection.

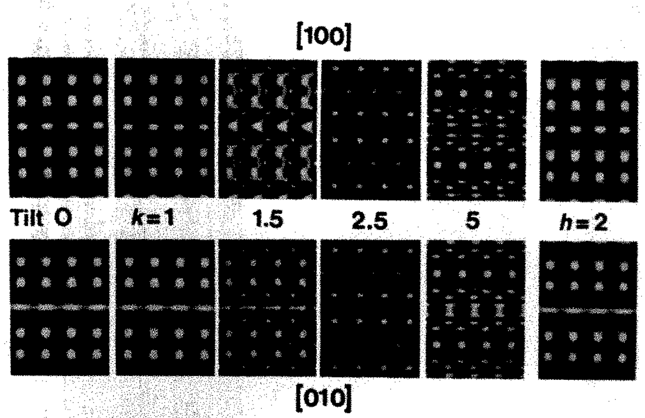

Fig. 4 Effect of tilt misalignment on lattice images in the [100] and [010] projections in the presence of perfect order. The centre of the Laué circle is at (h, k). Only the index specified has been changed from zero for each simulation; $h = 1$ corresponds to a tilt of ~4.3 mrad, with the centre of the Laué circle displaced towards the (100) or (010) spot, $k = 1$ to ~1.4 mrad, with the centre displaced toward the (001) spot. Beam divergence, 1 mrad; defocus, −500 Å; sample thickness, 100 Å. Note that tilt misalignments can remove the differences between the two projections (and also the ordered [010] and disordered configurations), but cannot mimic the effect of order in the [010] projection.

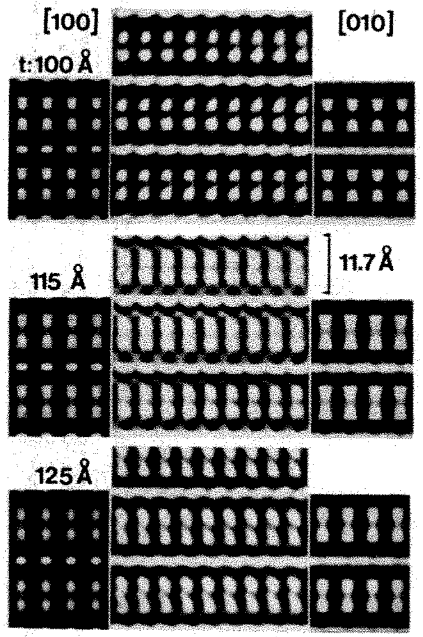

Fig. 5 Experimental images (centre) and simulations at three different thicknesses. Defocus, −500 Å; perfect oxygen order and alignment assumed in the simulations. It is likely that, experimentally, small tilt misalignments (≤1.2 mrad) may be present, or that the degree of order is <1. The experimental images nevertheless match the [010] ordered simulations much more closely and thus indicate the presence of order (of O-Cu-O chains parallel to the electron beam).

the electron beam direction. There are well-established procedures for the accurate alignment of the beam with respect to the optic axis.) The amount of tolerable misalignment depends on the sample thickness. For a film thickness of ~100 Å, misalignments greater than ~2.5 mrad, displacing the centre of the Laué circle towards the (001) spot, remove the differences between the [100] and [010] projections, and also between the ordered [010] and disordered configurations. For tilts displacing the centre of the Laué circle towards the (100) or (010) spots, however, larger deviations from exact alignment can be tolerated before the evidence for order is lost. Thus, tilt misalignments can change the character of the images and remove the possibility of detecting the presence of order, but, significantly, do not mimic the effect of order. Thus the experimental observation of images of the type shown in Fig. 3*c* and *d* provides important evidence for the absence of significant misalignment or oxygen desorption, as well as the presence of oxygen ordering.

In Fig. 5 we present experimental data on the thickness dependence of lattice images in regions with thicknesses and orientation appropriate for the detection of order. These images were obtained in a single exposure of a negative at the same defocus of −500 Å. The experimental defocus was first deduced to within ~10% by optical diffractometry on the neighbouring amorphous regions of the sample and further refined by fitting of simulated images to within ~20 Å. The thickness assignations were deduced by image matching to within 8 Å and checked by cross-calibration with the Pendellosung oscillations. The experimental images fit the ordered [010] images much more closely, and thus provide evidence for the presence of long-range order in the oxygen arrangement in the basal planes. By obtaining convergent beam electron diffraction patterns of this area and the neighbouring twins, we have independently established the projection to be [010], thus confirming the validity of our approach. In general, the white lines observed separating the basal planes show a modulation somewhat larger than present in the simulations. This could be due to the presence of an order parameter of less than one, produced during growth, or subsequently, due to oxygen desorption, and/or disordering during sample preparation. Additionally, or instead, small tilt misalignments may be responsible for the observed modulations of the white line. These observations emphasize the difficulty of quantifying the degree of order by lattice imaging, which might depend on an accurate determination of this modulation.

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Experimental evidence for convective rolls in finite two-dimensional molecular models

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Molecular dynamics (MD) techniques, based on explicit numerical solution of the equations of motion of the particles in a molecular system, have been successful in the study of models simulating fluids in equilibrium configurations as well as in stationary states due to the presence of external constraints. The possibility of observing true hydrodynamical behaviour has however been frequently questioned. There has been much recent interest[1] in new computational techniques which might be used to approach the difficult problems of flow and convection[2]. Results from studies of cellular automata[3,4] are very promising. Fluid flows obstructed by plates or cylinders have been studied, using MD techniques, by Rapaport and Clementi[5], who demonstrated the appearance of

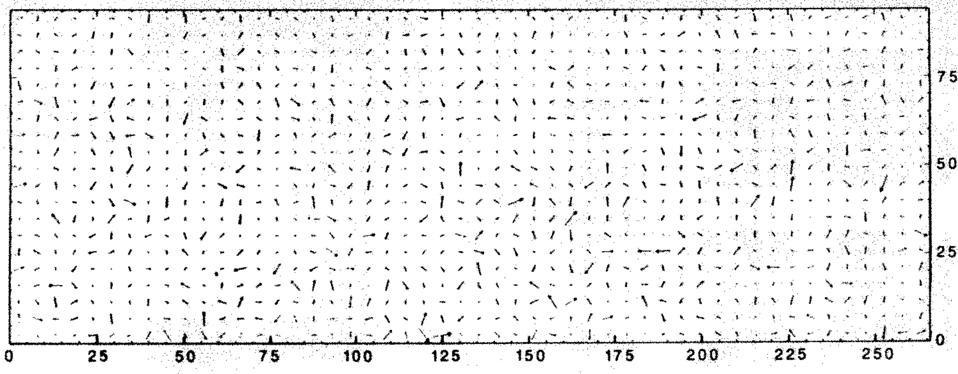

Fig. 1 Initial streamlines in a finite two-dimensional system of 5,040 hard disks under the influence of a thermal gradient and an external force. Number density is 0.2 and g is 0.09. Density velocities are normalized.

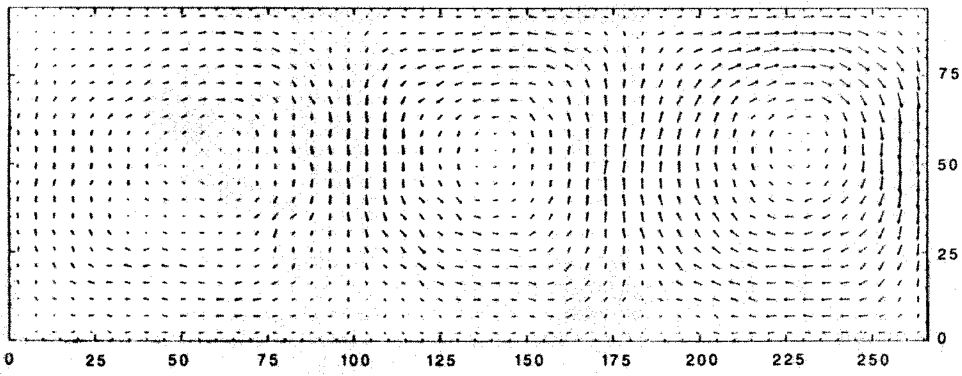

Fig. 2 Same system as in Fig. 1 after 13 million collisions. Rayleigh number is estimated to be 1,780. Each arrow is a time average of the local velocity density over the entire run.

vortices, although it was generally argued that such simulations were out of range of the power of present computers. Here, we study finite systems of hard disks[6] in the presence of both a thermal gradient and an external constant force. For well-chosen geometrical parameters, (density, gradient value and magnitude of the applied force), we observe the appearance of convective flow in these very small two-dimensional systems. Starting from a completely chaotic state, the system evolves to an ordered state after a few million collisions between particles. The vortices remain stable thereafter for at least a few thousand collisions per particle.

Our system consists of 5,040 hard disks placed in a rectangular box (L_x, L_z) at a number density of 0.2, the unit of length being the diameter of a disk. The dimensions of the box are respectively 266 in the x direction and 94 in the z direction. The $z=0$ boundary represents a reservoir at temperature $T_0+\Delta T$ while at $z=L_z$ temperature is maintained at T_0. Initially, the particles are placed at random in the system, their velocity components being chosen from equilibrium distributions at the local temperature extrapolated from a linear profile between the temperatures of the reservoirs. The boundaries of the system act as walls. On the x boundaries, particles undergo simple specular reflections. When a particle hits a thermal wall, its new velocity is sampled from the local distribution but the sign of the tangential (x) component is preserved. A constant external force acting in the opposite direction of the thermal gradient is applied on the molecules. In our simulations, the temperatures have been chosen as 1.0 and 10.0 respectively, and the constant external force corresponds to a gravitational acceleration of 0.09 in units where the mass of the particles, the molecular diameter and the Boltzmann constant k_B are 1.0. For the given number density, the measured temperature at $z=L_z/2$, and using the Enskog values[7] for the kinematic viscosity and thermal diffusivity, we estimate the Rayleigh number to be 1,780.

The system evolves in time according to our standard computer program, incorporating the principles described in ref. 8. To calculate local properties, the system is divided in 20×50 boxes, and from the chosen random initial condition is followed in phase space. After about one million collisions between particles (nearly 400 collisions per particle) we begin to compute at equal time intervals various mean quantities, for example, the local temperature (defined as the mean kinetic energy of the particles in the given box), the mean number density and the mean velocities of the particles.

We give in Fig. 1 a sketch of the initial mean velocity densities over a time interval equal to the mean collision time. As expected, mean velocities are random, and of the order of the square root of the local temperature. Figure 2 represents the same mean velocities, but after an evolution time of the order of a few thousand collision times. Vortices corresponding to a regular flow of particles from the warm bottom plate to the cold top one can now be seen. In this case, the mean velocities are one order of magnitude lower than the thermal velocities. Note that the linear temperature profile between the two plates is still present. Figure 3 gives this profile together with the variation of the local number density and local pressure in the system as functions of position. As time goes by, the structure becomes more and more pronounced, the asymmetry between the left and right sides of the system disappearing slowly. The vortices

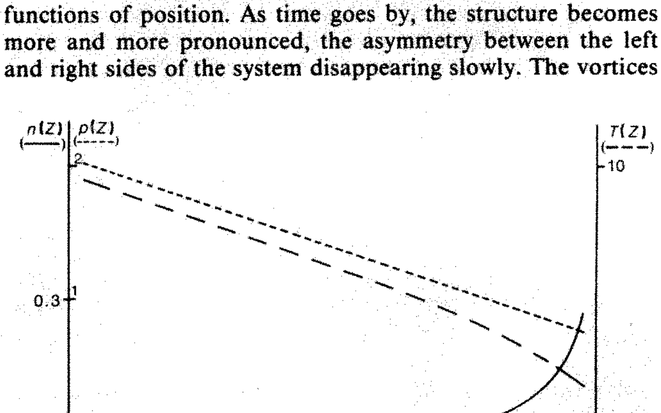

Fig. 3 Temperature, density and pressure profiles in the same system as a function of distance from the hot plate ($z=0$).

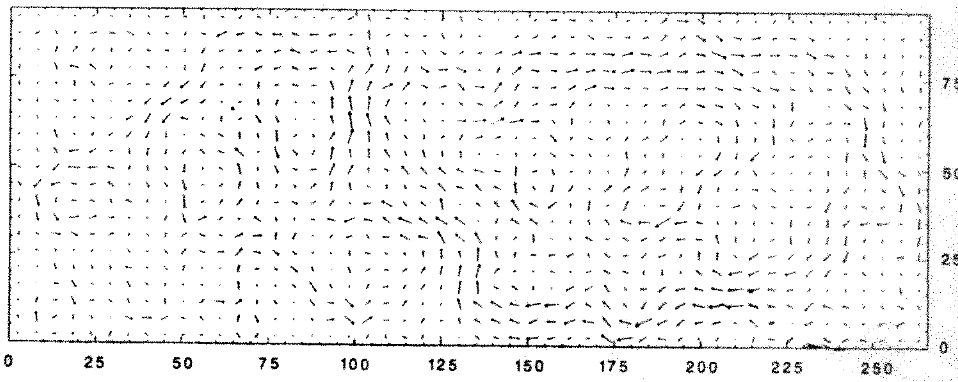

Fig. 4 Streamlines in a system of 5,040 particles with the same geometry and density as in Fig. 1, but with different values of the thermal gradient and of the gravitational acceleration, giving a Rayleigh number equal to 990.

remained stable throughout the experiment, which lasted for more than twenty million collisions (the computer program needed nearly 400 h of central processor time on a 4341 IBM computer); this corresponds to four times the time needed for heat to diffuse over a distance equal to L_z.

What is most surprising is the existence and persistence of such structures in very small systems. Most physicists were convinced of the need for systems two or more orders of magnitude bigger than this to observe such behaviour. It thus appears that the hypothesis of local equilibrium is verified on the microscopic level[8] and also that true collective motions exist for long, hydrodynamic times at dimensions close to a mean free path. The choice of the molecular and external parameters is crucial, as can be seen in Fig. 4, where we have drawn the flows for a system with the same number of particle and the same geometry, but with slightly different external force and temperature gradient. In this case, the Rayleigh number is reduced to 990, and, while whirlpools seem indeed to be present, there are no stable ordered structures. In particular, the computed histories of these model fluids show that the onset of an ordered state results from competition between spontaneous fluctuations giving rise to small structures and damping caused by their environment. Such a description falls outside the range of hydrodynamic equations.

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Mantle-derived helium in two Peruvian hydrothermal ore deposits

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Strong differences exist between the helium isotopic compositions of mantle-derived magmas[1–8], axial vent hotspring fluids[9–14], subduction-related magmas[15–20] and many associated hot springs[21–24], and the atmospheric and crustal values (Fig. 1). Here we report helium isotope ratios for two late Tertiary fossil hydrothermal systems responsible for precious-metal, base-metal and tungsten mineralization, and compare them to those of circum-Pacific hotspring emanations. The ^{3}He/^{4}He ratios of inclusion fluids hosted by gangue and sulphide minerals from Casapalca and Pasto Bueno, Peru, all lie between two and three times the atmospheric value, indicating the presence of a dilute component of mantle helium consistent with previously reported stable isotope evidence for the presence of magmatic fluids during mineralization[25–27]. Although post-trapping modification of the helium isotope composition is possible, we suggest that these apparently low values represent the composition of helium inherited from contributing magmas. As a tracer of magmatic fluids in hydrothermal systems, helium isotopes are decoupled from the factors that govern other stable isotope signatures.

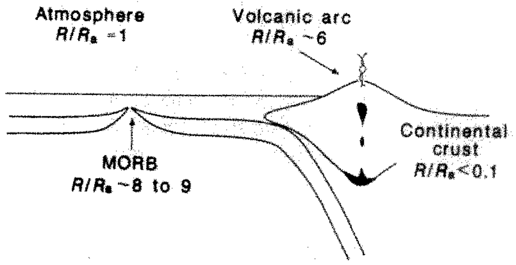

Fig. 1 Summary of helium isotopic compositions from various tectonic environments[36]. MORB, mid-ocean-ridge basalts.

Of particular interest are the many subaerial, circum-Pacific hot springs that exhibit strong mantle helium signatures, with the ^{3}He/^{4}He ratio (R) greater than six times the atmospheric ratio (R_a), but which have O and H isotope signatures representative of local meteoric water; for example, the Broadlands geothermal field, New Zealand[23,28]. For these hydrothermal systems, identification of mantle helium is evidence for the presence of a magmatic heat source at depth[23]. Crystalline igneous rocks are unlikely to contain sufficient amounts of helium available for hydrothermal leaching as most helium would have partitioned favourably into melt and gas phases during crystallization, for example, mantle helium signatures in young oceanic basalts are obtained from glassy rims of pillows, whereas the crystalline cores contain seawater (atmospheric) helium[2,29].

We have investigated the ^{3}He/^{4}He ratios in fluid inclusions from two well studied Andean metal deposits, Casapalca and

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Table 1 Temperature, salinity and stable isotope summary of mineralizing fluids

	Temperature (°C)	NaCl (% equiv. wt)	δD	$\delta^{18}O$	$\delta^{34}S$	$\delta^{13}C$
Casapalca	280–370	4–40	−48–−60	4.0–9.5	0.5–3.5	−6.5–−5.4
Pasto Bueno	175–290	2–17	−29–−88	0.0–7.8	−5.48–2.85*	−4.1–−11.9

From refs 25–27. * Pyrite data only.

Pasto Bueno, and these measurements add to their extensive fluid inclusion and isotopic databases[25-27]. Both deposits are closely associated with subduction-related magmatism, but are quite different in terms of their contained metals, host rocks and genesis. At Casapalca, steeply dipping Ag-Pb-Zn-Cu-mineralized veins crosscut a folded sequence of Tertiary sedimentary and volcanic units. Only minor amounts of intrusive rocks can be mapped at the surface in the district, but the occurrence of contact-metamorphosed and metasomatized rocks at depth below the vein system indicates the presence of an underlying igneous body. This interpretation is further confirmed by the clear magmatic signatures of the mineralization process provided by the light stable isotope data (Table 1). The final stages of mineralization were characterized by an influx of meteoric waters which deposited calcite.

The tungsten-base-metal-bearing veins at Pasto Bueno crosscut the contact between the Consuzo Stock, a late Tertiary quartz monzonite, and adjacent Mesozoic sedimentary rocks. An approximate age of 8 Myr is indicated from K–Ar dating of hydrothermal sericite. O, H, C and Sr isotope studies indicate: (1) fluorite was deposited from fluids of magmatic origin; (2) tungsten of magmatic derivation was deposited during influx of meteoric fluids; and (3) sulphide mineralization occurred in the presence of magmatic, meteoric and a third evolved water of unknown provenance[26,27].

Helium isotopic compositions of inclusion fluids trapped in quartz, sphalerite, tetrahedrite and fluorite range between two and three times the atmospheric ratio for both deposits (Table 2; Fig. 2). These measurements indicate that a portion of the helium in inclusion gases is mantle-derived and are consistent with previously cited stable isotope evidence for the presence of magmatic fluids during mineralization. However, the figures appear low, considering both the strong magmatic stable isotope signature for both deposits, and the high R/R_a values for many circum-Pacific hot springs that are clearly dominated by meteoric water. The possibility that helium isotope values are the result of lithium-nuclear reactions is discounted by the absence of any known lithium-bearing mineral in the vicinity of samples studied. Cosmogenic helium produced by spallation reactions at high elevations recently identified as a surficial phenomenon in the summit lavas at Maui[30,31] and in the Fresnillo District, Mexico[32] is also considered an unlikely source of ^3He as all samples were taken from underground workings, at depths greater than 100 m below the surface.

Possible explanations for the relatively low R/R_a values we report include: (1) preferential diffusive loss of ^3He from the fluid inclusions; (2) *in situ* generation of ^4He due to radioactive decay of U and Th which may be present as dissolved constituents in the host minerals or impurities in inclusion fluids; (3) fractionation of ^3He into the vapour phase during boiling, resulting in lowered concentrations of ^3He in liquid-rich fluid inclusions; or (4) dilution of mantle helium with crustal helium. This latter would be due to possible mixing with meteoric fluids in the hydrothermal system, magmatic assimilation of crustal material, or magma 'ageing' where radiogenic helium accumulates due to a long residence time within the magma chamber[33].

Diffusive loss is very difficult to evaluate and the problem may be compounded further if trace amounts (less than 1 p.p.m.) of U and Th are present in host minerals. Craig and Lupton[3] discussed these problems at length with respect to lower helium isotopic compositions in older basalt glass rims from pillow basalts. Although helium diffusivities in vesicular basalt glass and a mineral containing fluid inclusions are probably different their analysis shows that the residual R/R_a decreases logarithmically with respect to helium concentration. Only after more than 95% of the helium has been lost is the R/R_a half its inherited value, and thereafter the residual R/R_a becomes very sensitive to change in helium concentration. Accumulation of *in situ* radiogenic helium further enhances this sensitivity, therefore significant modification of helium concentration from the

Table 2 Summary of temperature, $\delta^{18}O$, δD, $\delta^{34}S$, $^{87}Sr/^{86}Sr$ and $^3He/^4He$ (R/R_a) data of inclusion fluids from Casapalca[25] and Pasto Bueno[26,27], Peru

Location and sample		Temperature (°C)	$\delta^{18}O$	δD	$\delta^{34}S$	$^{87}Sr/^{86}Sr$	$R/R_a \pm 1\sigma$	He($\times 10^{-6}$ cm^3 STP)	H_2O (mg)
Casapalca									
68-S-18	qtz (17 g)	359 (P), 336–356 (Ps)	3.8–5.4*				2.2 ± 0.1	9.0	13
68-S-30	sph (36 g)	354 (P), 323–333 (Ps)	7.3	−49–−65			2.1 ± 0.3	2.4	16.6
68-S-66	qtz (12 g)	307–327 (P & Ps)					2.3 ± 0.1	—	
69-S-7	sph (21 g)	331 (P), 300–303 (Ps)		−53	2.4		2.8 ± 0.2	1.3	
69-S-22	sph (24 g)				1.76		2.3 ± 0.2	1.5	8.7
Pasto Bueno									
9H-148A	wh fluor (19 g)	168 (P)	1.37*	−45.6			3.2 ± 0.2	5.1	9.2
9H-124	qtz (8 g)	150–180 (P)	2.20*	−35.8			2.1 ± 0.1	6.9	6.8
9H-150	fluor (14 g)	167-183 (P)		−46.8		0.70705	3.0 ± 0.2	5.1	
9Mn-126	tetr (22 g)				2.83		2.6 ± 0.1	12.5	7.2
9Mn-127	sph(31 g)				2.85	0.71228	2.7 ± 0.2	5.7	6.3
9H-152	tetr (20 g)			−113.1	−1.37		2.9 ± 0.2	8.3	
Blank							6.8 ± 5.8	0.075	

P, primary inclusions; Ps, pseudosecondary inclusions; qtz, quartz; sph, sphalerite; tetr, tetrahedrite; fluor, fluorite; wh, white.
* Calculated $\delta^{18}O$ composition of inclusion water.

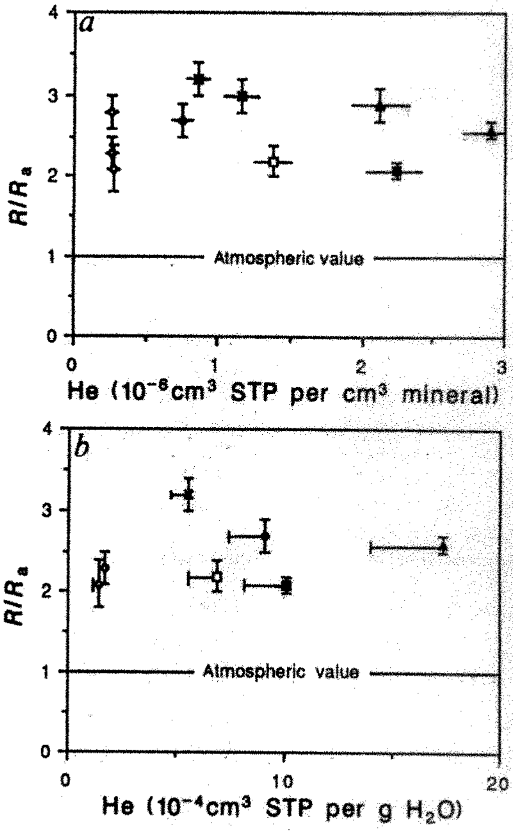

Fig. 2 Plots of R/R_a versus helium concentration treated either with respect to host mineral volume (*a*) or with respect to water released from fluid inclusions (*b*). Helium isotope compositions were measured from fluids trapped in 99% pure, mineral separates of quartz (qt), sphalerite (sp), tetrahedrite (td) and fluorite (fl). Samples were initially cut to less than 1-cm diameter with a water-cooled saw, ultrasonically cleaned in acetone and placed in the sample chamber where they were evacuated to less than 10^{-4} atm overnight. The following day, they were heated to >500 °C for 30 min to release gases by decrepitation of fluid inclusions. Helium and neon were separated from the other gases by a liquid N_2-cooled, activated charcoal trap and collected in a glass bulb. The bulb was immediately transferred to the inlet line of a 3.75-in radius double-focusing Mattauch–Herzog geometry mass spectrometer[37], dedicated to terrestrial samples only. The sample gas was further purified by a second liquid N_2-cooled, activated charcoal trap and a SAES getter, and then introduced into the mass spectrometer where ^{3}He and ^{4}He were measured simultaneously. Atmospheric standards were run before and after sample runs and averaged together to determine R/R_a reported in Table 2. Absolute amounts of helium reported were determined by comparing peak heights of known concentrations of helium in the air standards with the sample peak heights and are considered accurate to ±10%. Quantities of water released during decrepitation of samples were measured by weight difference after the water had been collected in a liquid N_2-cooled glass capillary. These are minimum values and are no more than 25% low. A procedural blank yielded 7.5×10^{-8} cm³ STP He and $R/R_a = 6.8$ (±5.3). *In vacuo* crushing experiments, although not used in the measurement of these samples, have been performed on hydrothermal quartz from underground mine workings in the Fresnillo district, Mexico, and have shown that the helium isotopic composition of gases released by this technique are identical to helium ratios measured of gas released by the thermal decrepitation technique[32].

original trapped concentration should be accompanied by noticeable changes in the R/R_a. Utilization of four different mineral species of different density, chemistry and crystallography suggest that diffusion rates, as well U and Th contents, for each of these minerals are different. All the Peruvian R/R_a values, however, fall within a narrow range despite the broad spread in helium concentration (Fig. 2), suggesting that diffusion and *in situ* ^{4}He production were not important in lowering the inherited R/R_a.

In situ radiogenic accumulation of ^{4}He could instead result from dissolved U within inclusion fluids. Although we lack data on U concentration in the inclusion fluids, a calculation can be made which gives a maximum ^{4}He production in 8 Myr from an assumed initial U concentration and the following equations[3]:

$$J(^4\mathrm{He}_R) = 0.2355\times10^{-6}\ \mathrm{U^*\ cm^3\ g^{-1}\ per\ 10^6\ yr}$$

where

$$\mathrm{U^* = U(p.p.m.)\,[1+0.123(Th/U-4)]}$$

Using a Th/U ratio of 0, given that thorium is highly insoluble[34], and a U concentration of 50 p.p.m. gives a total ^{4}He production of 4.8×10^{-5} cm³ STP per g H_2O in 8 Myr. Subtracting this amount of radiogenic helium from an intermediate helium concentration of 5.5×10^{-4} cm³ STP per g H_2O (sample 9H-148A) measured, gives an inherited helium isotopic composition of $R/R_a = 3.5$. This indicates that U concentrations far greater than 50 p.p.m. would be required to alter significantly the initial helium isotopic composition, a value that seems too high for fluids from ore-generating systems devoid of uranium minerals[35].

At Pasto Bueno, evidence of boiling exists only in the early stage of mineralization[26] and the minerals for this study came from later stage mineralization. Liquid-rich and vapour-rich inclusions were observed to coexist in Casaplaca sphalerite[25] and these samples should provide a ^{3}He/^{4}He composition closer to that of fluids prior to boiling. Consequently, it seems unlikely that preferential vapour-phase partitioning of ^{3}He could account for the same low helium isotopic compositions in both deposits.

Dilution of mantle helium in the hydrothermal system by a meteoric fluid containing radiogenic crustal helium could, in part, account for lower R/R_a at Pasto Bueno; however, this is not consistent with the strong magmatic O and H signatures at Casapalca[25].

Finally, the helium isotope data from these Andean ore deposits could represent the actual helium isotopic composition of fluids emanating from cooling intrusions at depth, an explanation we favour. This interpretation would imply that mantle helium signatures were modified by incorporating radiogenic helium within the magma before helium was released into the hydrothermal system, a process that could involve either crustal assimilation or magma 'ageing'. The similar values for both Casapalca and Pasto Bueno would suggest similar degrees of contamination as the magmas rose through the thick Andean crustal section. If this mechanism is important in controlling helium isotope signatures in magmatic-hydrothermal systems then intraoceanic arcs with thinner crust may have higher R/R_a values. We have preliminary data from the veins in the Baguio Gold Camp, Luzon (Philippines) which average $R/R_a \approx 6$, supporting this hypothesis. Unfortunately, active geothermal systems in the Andes have not been investigated with respect to helium isotope geochemistry, thus precluding a regional study of the effect of thick continental crust on magmatic-arc helium ratios.

In conclusion, mantle-derived helium, albeit diluted, has been identified in fluid inclusions associated with arc-related fossil hydrothermal systems, implying communication with a cooling intrusive body during mineralization. Further work is planned to investigate more thoroughly the possible mechanisms leading to apparent lowering of mantle helium signatures. Nevertheless, the uniformity of our data from a variety of minerals in two rather different deposits, one of which exhibits stable and strontium isotope evidence for three distinct fluid provenances, suggests constancy of source for helium and a decoupling from hydrological factors. This interpretation is further reinforced by the circum-Pacific hot-spring helium data where stable isotope evidence for meteoric waters is at variance with mantle helium isotope signatures.

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The relative importance of acidity sources for humic lakes in Norway

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The possible role of natural processes contributing to lake acidification and the occurrence of acidic lakes ($pH<5$) has received considerable attention[1–7], but there has been no direct test of the importance of some of the postulated natural processes in Scandinavia where large numbers of acidic lakes occur. Some authors have argued that lakes were acidic simply because of processes occurring in acid soils, and that the effects of acidic precipitation on these lakes were thereby overestimated[1,2]. We present here an analysis of the role of strong and weak acids in the acidity status of moderately humic lakes (total organic carbon (TOC) 2–15 mg l^{-1}) in two areas of Norway. The two areas received precipitation with differing acidity and amounts of sulphate. Our data suggest contributions by organic acids to acidity, but strong acids associated with sulphate further reduce the pH and explain the recent acidification of coloured lakes in southern Norway.

Dystrophic lakes, and especially bogs, may be naturally acidic due to the presence of organic acids[8–11]. Organic anions contribute to the acidity of lakes[12], but the relative roles of strong mineral acids and acidity associated with organic anions in lakes in areas containing large numbers of acidic lakes, such as southern Norway, are poorly described. A notable exception is the demonstration that both anthropogenic sulphur and organic acids contribute to the acidity of surface waters in Nova Scotia[12]. Palaeolimnological analyses based on diatom reconstructions have been presented as evidence for the acidification of lakes in Scotland, Norway and the northeastern USA associated with strong acids[13–17]. Diatoms have also been used to reconstruct historic concentrations of TOC; these results suggest some reduction in TOC during acidification[15].

Here we have assessed the relative contributions of acidity associated with organic anions and sulphate. To do this, we selected and sampled 94 lakes from two areas of Norway draining peaty soils and known to contain coloured lakes. The lakes in each area were located on granitic bedrock, were of similar size and had similar ranges of concentrations of base cations

Table 1 Minimum, mean and maximum values for selected chemical variables in lakes at Gulsvik (G) and Nord-Trøndelag (NT), Norway

Parameter	Gulsvik ($n=48$) min.	Gulsvik ($n=48$) mean	Gulsvik ($n=48$) max.	Nord-Trøndelag ($n=46$) min.	Nord-Trøndelag ($n=46$) mean	Nord-Trøndelag ($n=46$) max.
Conductance (μS cm^{-1})	1.18	2.01	2.58	1.60	2.82	4.51
Ca (mg l^{-1})	0.33	1.20	2.65	0.27	1.74	4.24
Mg (mg l^{-1})	0.10	0.23	0.39	0.28	0.54	1.09
Na (mg l^{-1})	0.28	0.56	1.23	1.10	2.70	5.30
Cl (mg l^{-1})	0.30	0.59	1.00	1.50	3.96	6.00
SO_4 (mg l^{-1})	2.50	3.31	4.50	1.20	1.69	3.10
NO_3-N (μg l^{-1})	1.0	17.7	62	1.0	11.7	81
TOC (mg l^{-1})	1.86	9.1	14.3	2.2	8.1	15.2
Anion deficit (μequiv. l^{-1})	−8.0	31.0	78	11.5	35.5	72.3
Ca* + Mg (μequiv. l^{-1})	21.4	75.4	146	16.2	105	244
SO_4^* (μequiv. l^{-1})	50.3	67.2	91.6	11.0	23.6	54.6
Na* (μequiv. l^{-1})	0.1	10.1	29.3	−19.5	16.5	45.9
Reactive Al (μg l^{-1})	56	168	490	13	57	172
Labile Al (μg l^{-1})	0	60	189	0	5	34
Strong acid (μequiv. l^{-1})	−38	7.2	37	−175	−67	5.5
Weak acid (μequiv. l^{-1})	49	129	253	72	116	185
Weak acid/TOC (μequiv. mg^{-1})	9.5	15.8	55.7	8.2	15.6	33.2

* Sea-salt corrected values. Weak acid, $40+2.9$ TOC $+0.6$ ILAL ($r^2=0.812$) (Gulsvik); $51+3.5$ TOC $+0.6$ ILAL ($r^2=0.823$) (Nord-Trøndelag).

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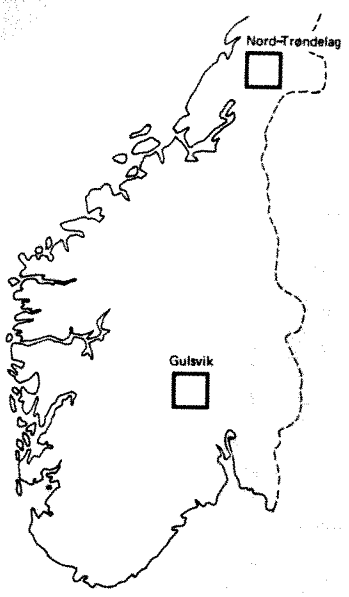

Fig. 1 Location of the Gulsvik and Nord-Trøndelag areas within Norway.

and TOC (Table 1), whereas mean base cations were lower in the lakes in southern Norway. The areas are separated by 550 km (Fig. 1). The area in southern Norway near Gulsvik receives precipitation with a lower *p*H (4.4 versus 4.9), higher concentrations of sulphate ($SO_4^* = 54$ versus 12 µequiv. l^{-1}) and lower contributions from sea salts (8.5 versus 68 µequiv. l^{-1} Cl^-) than the more northern area (Nord-Trøndelag), where * denotes sea-salt corrected values.

Samples were collected from lake outlets in the early autumn of 1986 and were analysed at NIVA[18]. Cations were determined by flame atomic absorption spectrophotometry and anions by ion chromatography. TOC was measured by a carbon analyser. Methods for aluminium measurements are given in ref. 19. Strong and weak acids were determined by an automated coulometric Gran titration[20].

The contributions of TOC and sulphate to *p*H were evaluated in three ways, including comparisons of the measured values of strong and weak acidity in the two areas. First, measured strong acids were significantly higher in the lakes near Gulsvik. Strong acids were negative for nearly all of the lakes in Nord-Trøndelag ($\bar{x} = -67$ µequiv. l^{-1}) and positive for most of the lakes in southern Norway ($\bar{x} = 7.2$ µequiv. l^{-1}), whereas concentrations of weak acids and the amount of weak acid per milligram of TOC were similar for both areas (Table 1). Second, in both areas TOC and non-labile aluminium explained more than 81% of the variance in weak acids with nearly equivalent non-labile aluminium (ILAL) coefficients for the regressions. The anion deficits (Σ Ca + Mg + Na + K + H $- \Sigma$ $HCO_3 + SO_4 + Cl + NO_3$) in both sets of lakes were also similar and related to TOC by: DIFF = 4.06 TOC − 0.96 ($r^2 = 0.551$) for all of the lakes. This relation is nearly identical to that in a recent survey of 1,000 lakes in Norway[21], suggesting that the organic anion relation in the lakes sampled by this study is representative of large numbers and probably the population of low Ca lakes located in the granitic areas of Norway. Third, to assess the contribution of sulphate and organic anions to acidity, we can examine lakes with comparable TOC and base cation concentrations in the two areas (Table 2). Data from individual lakes show that the differences in the concentrations of strong acid (or alkalinity) between the two areas are a function of the difference in sea-salt corrected sulphate (SO_4^*) in the lakes (Table 2). The concentrations of strong acids can be evaluated from measured values and separately estimated from the concentrations of hydrogen ions and labile aluminium. The lakes near Gulsvik contained on average 43.6 µequiv. l^{-1} more SO_4^* than did lakes in Nord-Trøndelag. TOC contributes to strong acids in both areas, but the higher SO_4^* in the lakes in southern Norway explains the difference in strong acid concentrations between the areas and the lower *p*H values for the lakes in southern Norway.

The difference in concentrations of sulphate determines the difference in the *p*H of lakes having equivalent concentrations of base cations and TOC. This is demonstrated for lakes in both areas having comparable concentrations of TOC and base cations (Σ Ca* + Mg* + Na* + K*). The data are presented for individual lakes, because the comparisons must be made in relation to concentrations of base cations and ion balance considerations. Strong acidity results when the sum of strong acid anions exceeds base cations. For lakes G-21 and NT-101, which have low concentrations of Ca* + Mg* (57 and 64 µequiv. l^{-1}) or total base cations and high TOC (11.5 and 12.4 mg l^{-1}), the difference in the concentrations of measured strong acids is equal to the difference in excess sulphate (Table 2). At even lower concentrations of base cations (samples G-70 and NT-107) (33 and 34 µequiv. l^{-1}) and TOC (5.6 and 8.8 mg l^{-1}), a similar difference is found in SO_4^*, which is only slightly higher than the difference in strong acids. At somewhat higher concentrations of base cations (54 and 54) but low TOC (2.7 and 3.8) (G-26 and NT-134), the total difference in strong acidity is also equivalent to the difference in SO_4^*. This is also true at higher Ca* + Mg* (68 and 68) and TOC (8.7 and 9.4) (G-68 and NT-136). In addition, the concentration of strong acids is higher at constant SO_4^* with lower Ca* + Mg*, because in the Gulsvik area, strong acids = 7.97 − 0.84 Ca^{2+} + 4.28 TOC − 0.15 weak acids + 0.44 SO_4^* ($r^2 = 0.864$; units are µequiv. l^{-1} except TOC as mg l^{-1}). Thus, base cation concentrations regulate sensitivity, whereas SO_4^* and organic acids control *p*H. For all the lakes, strong acids = −25.78 + 2.83 TOC + 0.96 SO_4^* − 0.98 Ca^{2+} ($r^2 = 0.948$). We can conclude that with TOC and base cations held constant, the difference in strong acids between the areas is

Table 2 Comparisons of measured strong acidity and *p*H for lakes in two areas of Norway

Lake number	*p*H	Conductance (µS)	ALK (µequiv. l^{-1})	TOC (mg l^{-1})	DIFF (µequiv. l^{-1})	Strong acid (µequiv. l^{-1})	Weak acid (µequiv. l^{-1})	Cl (mg l^{-1})	NO_3-N (µg l^{-1})	RAL (µg l^{-1})	LAL (µg l^{-1})	Ca* + Mg* (µequiv. l^{-1})	SO_4^* (µequiv. l^{-1})	Na* + K* (µequiv. l^{-1})
G-21	4.50	2.36	—	11.5	26	26	141	0.50	6	147	37	57	71	7
NT-101	5.62	3.04	18	12.4	56	−22	167	5.1	7	172	16	64	23	30
G-70	4.48	2.22	—	5.6	7	35	65	0.3	2	89	49	33	66	6
NT-107	5.32	2.24	5	8.8	37	1	130	3.8	6	107	17	34	19	21
G-68	4.65	2.32	—	8.7	35	25	139	0.6	10	204	98	68	63	7
NT-136	6.13	2.72	24	9.4	42	−30	147	4.8	2	97	5	68	15	9
G-26	5.37	1.36	—	2.7	8	−9	103	0.4	53	171	111	54	66	16
NT-134	6.27	2.28	27	3.8	20	−47	94	4.0	22	29	4	54	18	9
G-4	5.01	2.02	—	11.3	55	−6	157	0.6	1	213	55	108	77	14
NT-133	5.91	3.13	34	13.8	72	−54	185	5.2	2	114	7	103	20	15
NT-140	6.66	2.94	73	4.3	17	−61	79	4.8	—	16	1	95	21	10

G, Gulsvik; NT, Nord-Trøndelag; DIFF, (Ca + Mg + K + Na + H) − ($HCO_3 + SO_4 + Cl + NO_3$); RAL, reactive aluminium; LAL, labile aluminium.

about equivalent to the difference in excess sulphate, and TOC contributes some strong acids in each area.

The organic contribution to acidity can be estimated from lakes NT-133 and 140 (Table 2). These lakes have similar Ca^*+Mg^* (103 and 95) and equivalent $SO_4^{=}$ but differ in TOC by 9.5 $mg\,l^{-1}$ (13.8 versus 4.3). The higher TOC lake (NT-133) has a lower *p*H (5.91 versus 6.66). Normalizing to equal base cation concentrations, the difference in alkalinity (48 μequiv. l^{-1}) corresponds closely to the difference in anion deficit between the lakes, thus explaining the difference in *p*H. The anion deficit (unmeasured organic anions) is 72 μequiv. l^{-1} compared with 17 μequiv. l^{-1} for the low TOC lake. This contribution is 5.5 μequiv. mg^{-1} TOC, close to the mean value for the sample of 1,000 lakes[21]. This pair of lakes demonstrates that organic anions can contribute significantly to lakewater acidity at approximately 4–5 μequiv. mg^{-1} TOC. While organic anions contribute to acidity, the high TOC lake maintains a *p*H well above levels toxic to brown trout.

One site in Nord-Trøndelag showed evidence of a *p*H depression due to the sea-salt effect[22,23]. This was a small shallow pond draining a *Sphagnum* lawn. The moss effectively exchanged incoming Na^+ for H^+ ($Na^* = -19.5$ μequiv. l^{-1}; $Cl^- = 155$ μequiv. l^{-1}) and the *p*H was 4.84. This bog lake was the only site that showed such an effect even though the contribution of sea-salt is relatively high in Nord-Trøndelag. The sea-salt effect typically produces only episodic *p*H depressions, and is restricted to coastal areas[22-24]. This effect did not contribute measurably to the acidity status of the lakes in either area.

We have shown that both organic acids and $SO_4^{=}$ contribute to strong acids. However, there is no evidence for a significant increase over time in the contributions of organic anions in lakes in southern Norway that would explain decreases in *p*H. It is therefore unlikely that organic anions have contributed to the recent significant decreases in lakewater alkalinity (acidification) and *p*H observed in southern Norway. The dark-water lakes in the Gulsvik area, although having sources of TOC in their watersheds, have anions dominated by $SO_4^{=}$ similar to clearwater acidic lakes in southernmost Norway[25]. The organic acids make humic lakes more sensitive to acidic inputs from the atmosphere than clearwater lakes, for lakes with the same concentrations of base cations. This is also indicated by data from lakes in Nova Scotia[12]. Organic acids originating from watersheds contribute to the acidity of the lakes, but not necessarily to the recent acidification of lakes. Rather, the higher values of strong acidity in southern Norway correlate with higher excess sulphate, because strong mineral acids are superimposed on organic contributions to acidity. The differences in strong acidity between lakes of comparable watershed factors and chemistry found by the present study are associated with the pattern of sulphate concentrations in lakes and the difference in precipitation acidity. This implies that the recent acidification of lakes in southern Norway, both clearwater lakes and those with TOC concentrations up to 15 $mg\,l^{-1}$, is a result of atmospheric sources of acids and not natural processes.

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The age of the British chalk grassland

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Tansley[1] erected the hypothesis that the grasslands of the British chalk were the product of neolithic human forest clearance although he did not discount the possibility that in some areas the grasslands might have persisted throughout the forest period. Pigott and Walters[2] suggested a range of calcareous habitats where forests perhaps failed to form a closed canopy: where erosion was rapid, slopes steep, or soils too thin for trees to root. Despite these exceptions, most authors describe chalk grasslands as the product of neolithic clearance (for example, refs 3 and 4). The debate has been starved of evidence until now by the almost total lack of Flandrian botanical data from sites actually on the chalk. Here we summarize the results of a broad palaeoecological study (using pollen, and plant and animal macrofossils) of a site in the heart of the Yorkshire Wolds which has yielded the first late-glacial and early Flandrian pollen record from the British chalk[5]. This deposit has provided evidence of early Flandrian chalk grasslands and of their persistence throughout the Pre-Boreal and Boreal periods.

Willow Garth is a 5-hectare carr lying in the Great Wold Valley (grid reference TA 126676) at an altitude of ~18 m Ordnance Datum. The woodland is flanked by arable fields which, when ploughed, reveal a patchwork of peaty and sandy deposits. To the north, Willow Garth is bounded by an underfit stream, the Gypsey Race[6], which when it floods causes a pool to lie on the Willow Garth for some days. The carr has a more regular supply of water from at least five springs in the wood.

After an extensive stratigraphical survey (64 bore-holes and two ditch sections) the point of deepest organic accumulation (1.18 m) was identified (Fig. 1). This was a wet hollow ~20 × 30 m which supported a growth of *Iris pseudacorus*, *Filipendula ulmaria* and *Phalaris arundinacea*, shaded by *Salix fragilis*. The material for examination was collected from this point and was found to consist of 7 cm of undecomposed organic matter below which lay 41 cm of gyttja. From 48 cm to 118 cm there was a moss-peat, in which *Amblystegium riparium*, *Calliergon* spp., *Drepanocladus* spp. and *Scorpidium scorpioides* were the dominant taxa. Within this peat was a wood-rich band at ~76–82 cm. The basal contours of the peat were plotted and it was found that the sides of the hollow sloped gradually towards the

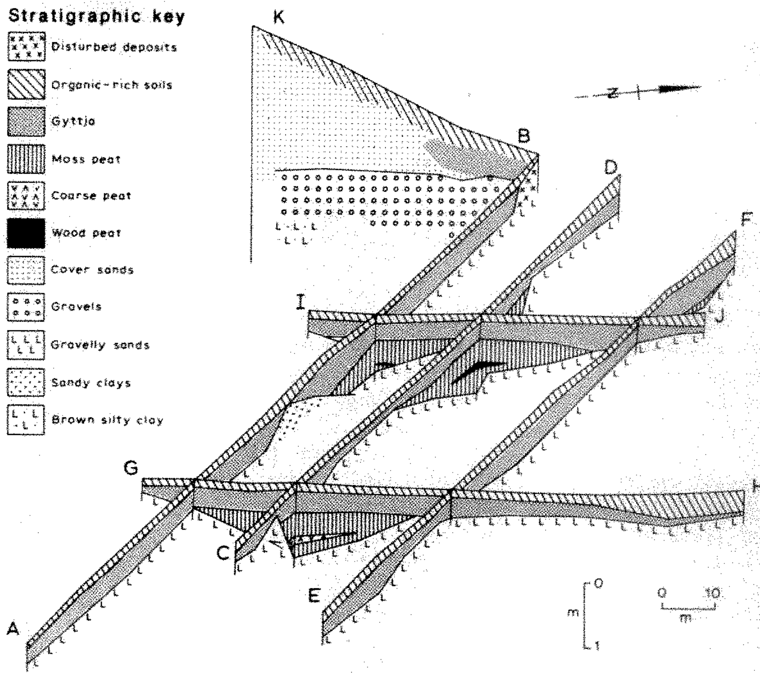
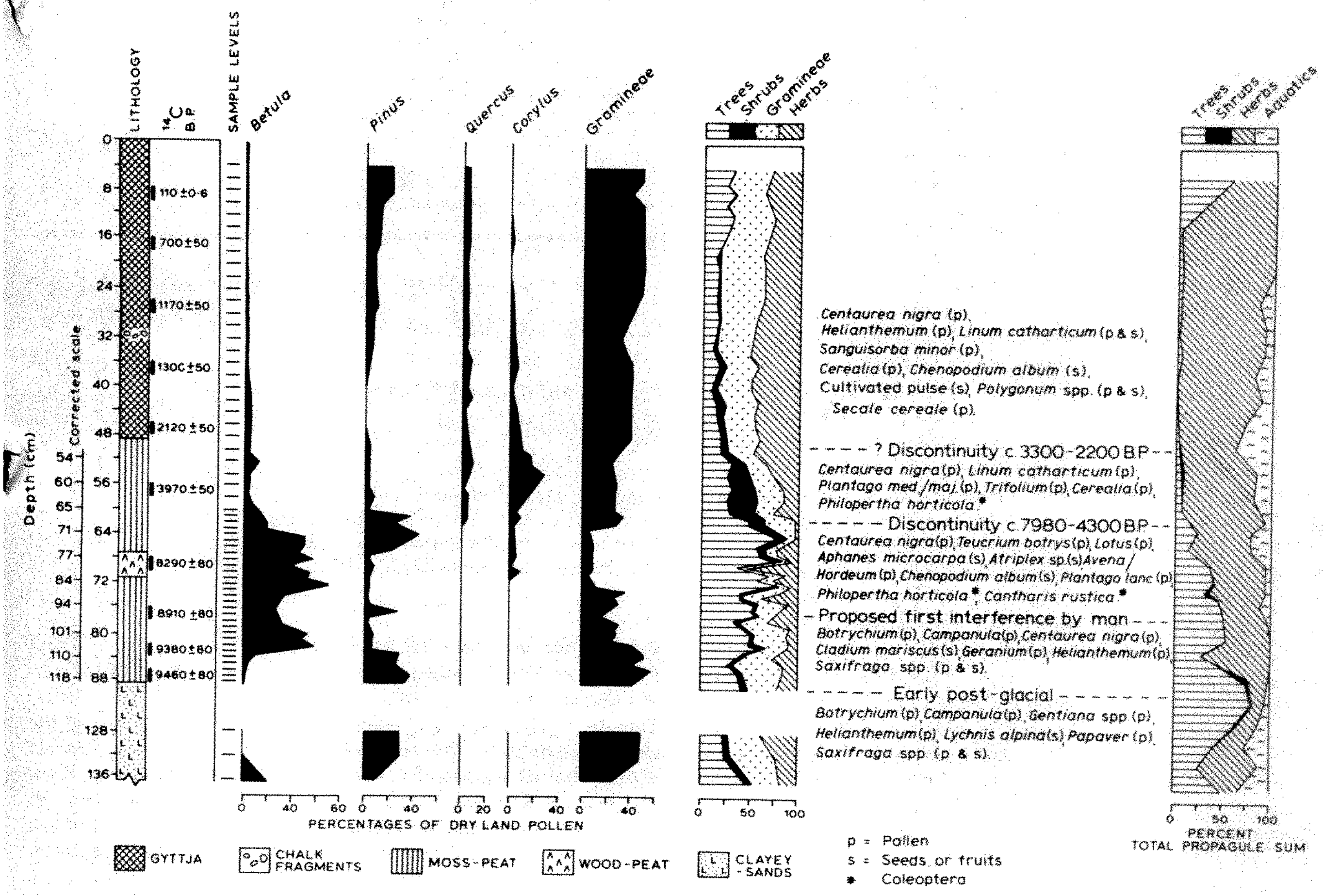


Fig. 1 (right) The stratigraphy at Willow Garth. The core for analysis was collected at the intersection of transects CD and IJ.

Fig. 2 (below) Summary pollen diagram from Willow Garth. Only selected taxa are shown.



Centaurea nigra (p), Helianthemum (p), Linum catharticum (p & s), Sanguisorba minor (p), Cerealia (p), Chenopodium album (s), Cultivated pulse (s), Polygonum spp. (p & s), Secale cereale (p).

– – – – ? Discontinuity c. 3300–2200 B.P. – –

Centaurea nigra (p), Linum catharticum (p), Plantago med./maj. (p), Trifolium (p), Cerealia (p), Philopertha horticola*

– – – – – Discontinuity c. 7980–4300 B.P. – –

Centaurea nigra (p), Teucrium botrys (p), Lotus (p), Aphanes microcarpa (s), Atriplex sp. (s) Avena/Hordeum (p), Chenopodium album (s), Plantago lanc (p), Philopertha horticola*, Cantharis rustica*

– Proposed first interference by man – – –

Botrychium (p), Campanula (p), Centaurea nigra (p), Cladium mariscus (s), Geranium (p), Helianthemum (p), Saxifraga spp. (p & s).

– – – – – Early post-glacial – – – – – – –

Botrychium (p), Campanula (p), Gentiana spp. (p), Helianthemum (p), Lychnis alpina (s), Papaver (p), Saxifraga spp. (p & s).

p = Pollen
s = Seeds or fruits
* Coleoptera

centre. Sands with a low organic component lay beneath the peat (Fig. 1). Generally, these were coarse to gravelly sands, although those at the sample site were clay-rich (Fig. 2).

Using a Livingstone piston sampler (7 cm diameter) samples were collected for pollen analysis and radiocarbon dating. A monolith for macrofossil analysis was extracted from within 0.5 m of the pollen site. The pollen core suffered some compaction during collection but this could be corrected for, because a trial core, taken alongside using a D-section borer, showed the same total depth and stratigraphy but was not compressed. All depths referred to in the text are 'corrected' depths.

Preparation of the pollen samples was by treatment with potassium hydroxide and acetolysis[7], and by the use of 10-μm ultrasonic filtration[8]. Macrofossils were extracted as in ref. 9. Pollen counts had a range of 300–750 dry land pollen. Macrofossil counts were to a minimum of 200 higher plant propagules.

Ten radiocarbon dates were obtained (SRR-2665 to 2674, Fig. 2). The samples were examined, rootlets removed before dating, and only the 'organic carbon' used to establish ^{14}C dates.

Old carbonate error does not appear to have been a problem as the wood-peat horizon at 76–82 cm offered a date consistent with those from the moss-peat and $\delta^{13}C$ values are normal. The base of the peat was dated at 9,460 ± 80 BP; however, the sandy material underlying the peat is, on the basis of pollen and macrofossils, estimated to have been deposited towards the end of the Windermere interstadial. The deposit seems to cover the period ~11,000 yr BP to ~7,980 yr BP (67 cm) when there was a standstill level. The record continued from ~4,300 yr BP through to the present day, although there may have been a further standstill level between ~3,300 and 2,200 yr BP.

In the basal sediments there is clear evidence of a cold-climate grassland flora with such species as *Gentiana verna*, *Diphasium alpinum*, *Lychnis alpina*, *Dryas octopetala*, *Saxifraga oppositifolia*, *Armeria maritima*, and *Plantago coronopus* (Fig. 2). Between ~9,380 and 9,300 yr BP *Betula* (probably *B. pubescens*) increased from ~10% to 58% of the dry land pollen count. However, this expansion was not maintained and *Betula* failed to exclude the open grassland taxa such as *Helianthemum*, *Campanula*, *Plantago coronopus* and *Botrychium lunaria*.

Betula declined to 24% (97 cm) and there was a peak of *Pinus* at 93 cm, immediately preceding the first regular occurrence of pollen and macrofossils of temperature arboreal taxa, such as *Corylus*, *Sambucus* and *Quercus*. However, these new arrivals failed to gain any kind of dominance and *Betula* showed a second peak of 68% at 84 cm.

Throughout this period there were high Gramineae levels, mostly 20 to 33% dry land pollen when, in most other British pollen diagrams, ~5% would be expected[10]. Grassland species had also persisted with *Plantago media* and/or *major*, *Campanula*, *Vicia*, *Lotus* and *Centaurea nigra* all present.

The failure of the temperate woodland taxa to dominate the pollen spectrum and the re-emergence of *Betula* suggests that the normal process of colonization had been halted. This does not seem to have been due to fire as there was no evidence of charcoal inwash, nor of peaks of fire-resistant taxa such as *Corylus*. The timber-rich layer of *Alnus*, *Salix* and *Quercus* wood suggests that there may have been some felling of the local forest. Although the two former species may have grown on the fen and collapsed into it, it is less likely that *Quercus* would have done so. The sudden occurrence of macrofossils of *Chenopodium abum*, *Atriplex* cf. *patula*, *Aphanes arvensis*, and *Stellaria media* would support the suggestion that forest disturbance was taking place. Further evidence of disturbance was provided by the occurrence of insects characteristic of open grassland, such as *Philopertha horticola*, which was in every sample between 84 cm and 64 cm, *Cantharis rustica* and *Serrica brunea*. These insects all need dry grassland either in their immature or adult forms. The consistent presence of *P. horticola* is strongly suggestive of clearance (H. K. Kenward, personal communication). From this evidence it is suggested that man was disturbing the forest around Willow Garth from the beginning of the recovery of *Betula* (~8,900 yr BP). It is suggested that the resurgence of *Betula* in these circumstances would be as a secondary growth tree, or one which was able to flower despite cutting.

Cutting the trees would have had the effect of increasing young shoots for grazing wild cattle, could have increased the biomass of ungulates grazing the land by as much as 10-fold[11], and would have provided man with seeds from such plants as *Chenopodium album*, *Atriplex* cf. *patula* and *Avena*. Seven Gramineae pollen of >44 μm length were recovered from four samples between 93 and 88 cm (maximum size 52 μm with 10.5 μm annulus). These were compared with a range of reference material and identified as cf. *Avena*/*Hordeum* (presumably wild varieties).

Through this kind of management (forest clearance and grazing) the grasslands would have prospered as indicated by the presence of such taxa as *Teucrium botrys*, *Centaurea nigra* and *Sanguisorba minor*. The latter was found at a corresponding level in a preliminary core, though not in the main pollen core.

No palaeoecological evidence is available from this site between ~7,980 and 4,300 yr BP, hence it is not possible to state whether these grasslands were the same as those which were evidenced above the standstill level. Open chalkland landscapes in early neolithic times (before 4,800 yr BP) have been described, for example at Kilham[12]. Our results demonstrate the capacity of chalk grasslands, with the help of man, to survive the forest closure of the Pre-Boreal and Boreal periods, even in an area of gentle slopes. This leaves a period lasting from ~7,980 to 4,300 yr BP when the fate of the chalk grasslands has yet to be ascertained. Given the density of mesolithic and neolithic settlement in the Great Wold Valley[13], it is likely that forest disturbance would have continued throughout this period. From 4,300 yr BP to the present the existence of grasslands is clear from Fig. 2. Hence, there is a strong probability that, in at least some areas of Britain, the chalk grasslands have been a continuous feature of the post-glacial landscape.

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Upper Palaeolithic boomerang made of a mammoth tusk in south Poland

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Excavations in a cave in the Obłazowa Rock in south Poland revealed an Upper Palaeolithic cultural level with an abundance of lithic and organic artefacts as well as palaeozoological material. This level yielded a fragment of mammoth tusk showing the characteristic traits of a complete boomerang. This is the earliest certain find of this type of weapon in the world. The same layer also contained an adult distal thumb phalanx which represents the earliest find of human skeletal material in Poland to date. Human food debris in the cultural layer suggests that the Upper Palaeolithic people inhabiting the cave hunted reindeer for the most part. The faunal complex of this cultural level is typical of the Upper Pleistocene steppe–tundra, while the artefacts belong within the complex of Central European cultures with backed points and have attributes which allow them to be grouped within the Pavlov culture dating from ~23,000 yr BP. The site might have been of importance in the course of migration of human groups with backed points from today's Moravia and southwestern Slovakia regions north-eastwards to the Don river basin.

Several definitions of the boomerang variously based on criteria such as material, purpose, aerodynamic qualities and shape occur in the vast literature on this weapon. Generally

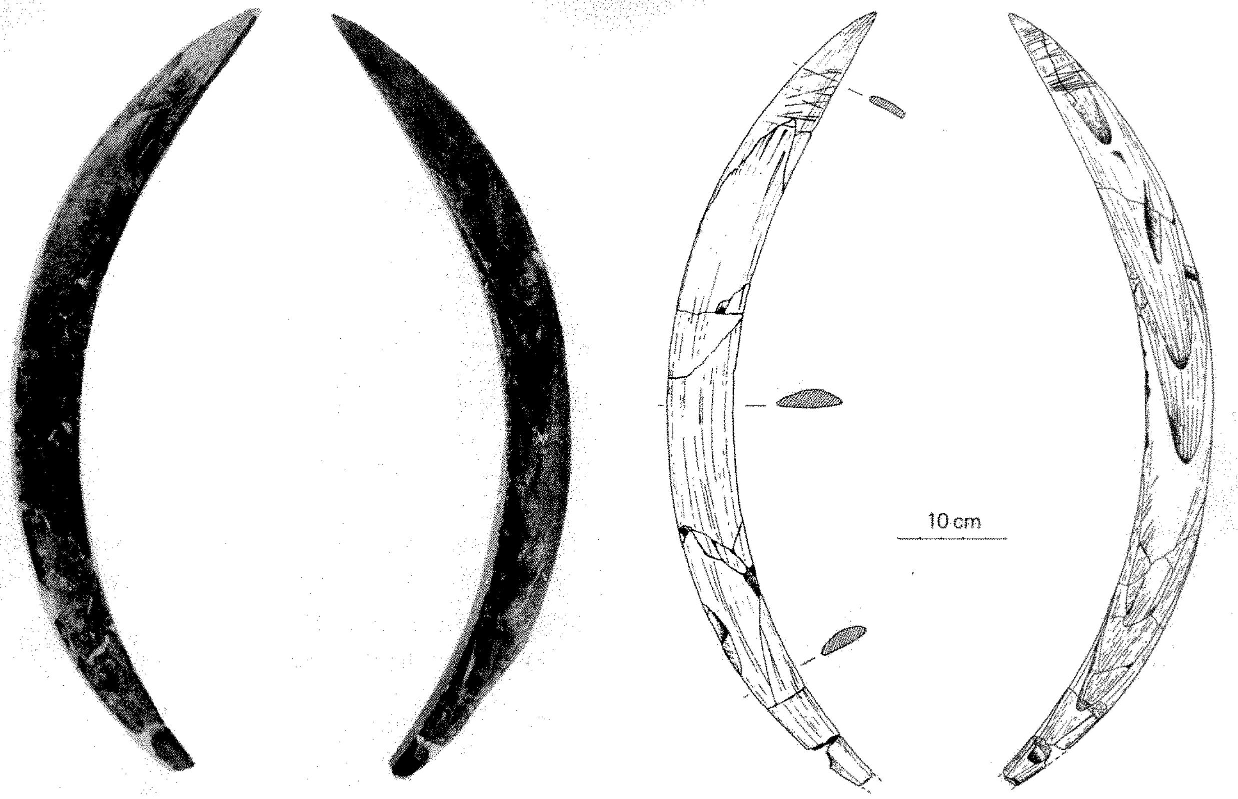

Fig. 1 External (left) and internal (right) views of a fragment of mammoth tusk interpreted as a boomerang.

only returning specimens are recognized as boomerangs, others being excluded as 'killing sticks'[1,2]. In this account we have adopted the definition presented in the latest monograph by H. Peter[3] which is based on criteria of form (curvature and bilateral flattening), rather than 'returning ability', which may vary due to possible manufacturing faults, damage or deformation, and is impossible to verify in an archaeological specimen.

Today the boomerang is prevalent only in the Australian Aborigine culture, but in ancient times it was used on virtually all inhabited continents[3]. In Europe the Iron age site of Velsen in the Netherlands[4] and the Mesolithic Brabrand-Sø in Jutland[5] are known for discoveries of wooden boomerangs. At Stillfried in Lower Austria a mammoth bone fragment resembling a fragmented boomerang was found[6] in an Upper Palaeolithic context but the exact chronology and culture are not certain[7]. The present discovery of a complete boomerang in Poland is thus the oldest definite find of this kind of weapon.

In summer 1985 the Institute of Systematic and Experimental Zoology, Polish Academy of Sciences, and the Department of Archaeology of Little Poland, Institute of the History of Material Culture, Polish Academy of Sciences, started excavations in a cave in the Obłazowa Rock in south Poland (49°25′48″ N, 20°9′36″ E). The cave is located in the lower part of the Obłazowa Rock (670 m above sea level) which, together with the Kramnica Rock (688 m above sea level), constitutes the inlet to the Białka river gorge. The cave originated through decay and water erosion of a tectonic breccia in the red nodular limestone[8,9]. A relatively small arched entrance to the cave, almost completely silted up before the excavations, is situated in the south wall of the rock about 6 m above the recent valley of the Białka river. The greatest length of the cave exceeded 9 m while the breadth was over 4 m. About 12 m^3 of the deposits has so far been excavated, resulting in exposure of the greater part of the entrance. However, to date the rock floor has not been detected.

Excavations have so far revealed nine sedimentary units containing abundant palaeozoological material and three Upper Palaeolithic cultural levels. The lowest of these levels has proved the most abundant in artefacts and contained part of a mammoth tusk which exhibits the characteristics of a boomerang (Fig. 1). The span of the boomerang is about 70 cm, the greatest breadth 6 cm, and the greatest thickness about 1.5 cm. Its convex face is in fact the external tusk surface and exhibits some scratches which probably originated during the animal's life. On the other side it is almost flat as a result of polishing and shows several scratched lines without any decorative pattern, roughly parallel to the longer axis of the specimen. At one of its ends tiny transversal lines are visible on both sides. These lines are not decorative and presumably relate to the manufacture of the

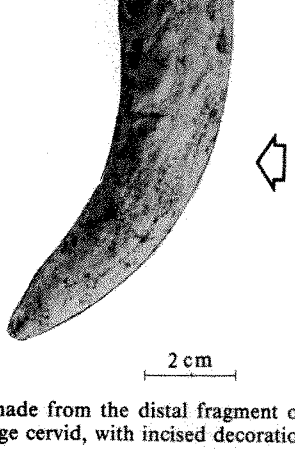

Fig. 2 Wedge made from the distal fragment of an antler of a large cervid, with incised decoration.

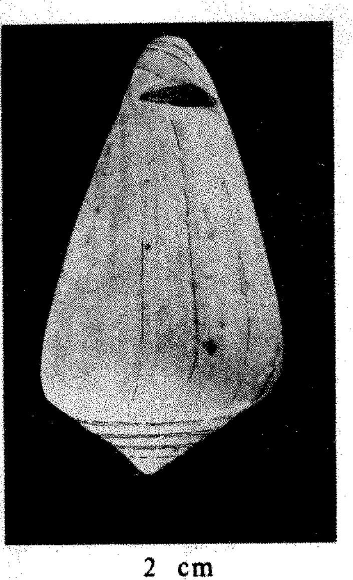

Fig. 3 Shell of a snail (*Conus* sp.) with an intentional aperture at the basal end. Assumed to have been used as bullroarer or pendant.

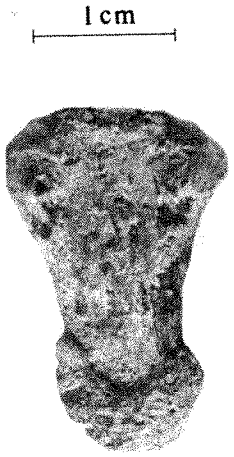

Fig. 4 Ventral view of the adult distal left thumb phalanx.

piece. Red colouring from a mineral substance was detected on some parts of the weapon, but there is no evidence to suggest that this is a result of painting.

Apart from the boomerang this lowest cultural level contained seven other artefacts made of organic materials. These include: a wedge made from the distal fragment of the antler from a large cervid, with a sharpened working end and decoration consisting of a few parallel arcuate lines (Fig. 2); a pendant or possible bullroarer made of a snail shell (*Conus* sp.) (Fig. 3); a necklace element made of the upper canine of an Arctic fox (*Alopex lagopus*); a perforator; and three bone beads. The assemblage of 30 stone artefacts comprise two flint cores and one of radiolarite, some blades, flakes and tools. Among the tools is an oblique truncated blade with undulating truncation (of Rgani type), a short end-scraper and a large tool of a cleaver type. The remains of a structure which consisted of several large granite and sandstone boulders up to 0.5 m in diameter were also found in this layer. Finally this cultural level also yielded the distal phalanx of an adult left thumb (Fig. 4), which represents the earliest find of human remains in Poland, as well as the bones and teeth of amphibians, reptiles, birds, and mammals, including Arctic lemming (*Dicrostonyx gulielmi*), Norway lemming (*Lemmus lemmus*), souslik (*Spermophilus* sp.), root vole (*Microtus oeconomus*), narrow-skulled vole (*Microtus gregalis*), snow vole (*Microtus nivalis*), pika (*Ochotona* sp.), hare (*Lepus* sp.), wolf (*Canis lupus*), Arctic fox (*Alopex lagopus*), stoat (*Mustela erminea*) and reindeer (*Rangifer tarandus*).

The faunal material from the cultural level containing the boomerang is not homogeneous in origin. The abundant fauna of small vertebrates undoubtedly derive from owl pellets, while some of the larger bones may represent food remains of other animal inhabitants of the cave as well as the bones of animals which died inside the cave. There is relatively little human food debris and this comprises heavily split reindeer bones for the most part, suggesting that the Palaeolithic people living in this cave hunted mainly this ungulate. The whole faunal complex indicates a cold period of the last glaciation when the extensive steppe-tundra covered the cave environs.

In general the artefacts of the lowest cultural level in question can be related to the complex of Central European cultures with backed points, showing traits of the Pavlov culture which is about 23,000 yr BP. The cultural characters of the inventory and the geographical position of the cave suggest that this site might have been of importance in the course of migration of human groups with backed points from today's Moravia and south-western Slovakia north-eastwards to the Don river basin[10].

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Endstopped neurons in the visual cortex as a substrate for calculating curvature

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Neurons in the visual cortex typically respond selectively to the orientation, and velocity and direction of movement, of moving-bar stimuli. These responses are generally thought to provide information about the orientation and position of lines and edges in the visual field. Some cells are also endstopped, that is selective for bars of specific lengths. Hubel and Wiesel first observed that endstopped hypercomplex cells[1] could respond to curved stimuli and suggested they might be involved in detection of curvature, but the exact relationship between endstopping and curvature has never been determined. We present here a mathematical model relating endstopping to curvature in which the difference in response of two simple cells gives rise to endstopping and varies in proportion to curvature. We also provide physiological evidence that endstopped cells in area 17 of the cat visual cortex are selective for curvature, whereas non-endstopped cells are not, and that some are selective for the sign of curvature. The prevailing view of edge and curve determination is that orientations are selected locally by the class of simple cortical cells[3] and then integrated to form global curves. We have developed a computational theory of orientation selection[4,5] which shows that measurements of orientation obtained by simple cells are not sufficient because there will be strong, incorrect responses from cells whose receptive fields (RFs) span distinct curves (Fig. 1). If estimates of curvature are available, however, these inappropriate responses can be eliminated. Curvature provides the key to structuring the network that underlies our theory and distinguishes it from previous lateral inhibition schemes[6,7].

The defining characteristic of endstopped cells is the presence of inhibitory receptive field (RF) end zones that 'stop' the response of the cell to stimuli which are long enough to intrude into the end zones. Two alternative models of endstopping have been proposed[1,2]: in one, the inhibitory 'endzones' derive from two or more independent cortical afferents with offset RFs; in the other, they derive from a single inhibitory input with a long RF. Both are compatible with the requirement that the endzones

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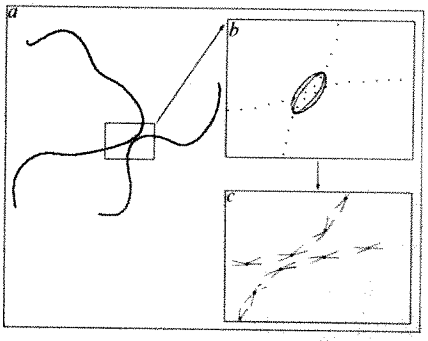

Fig. 1 Illustration of the use of curvature information in orientation selection. *a*, Two distinct curves closely approach each other so that the tangent to a point on one of the curves is colinear with the tangent to a nearby point on the other curve. *b*, This region is schematically expanded. In simulations, a simple cell-like operator centred between the curves and spanning both is strongly activated. This leads to an incorrect representation of the two distinct arcs as a single, unresolved curve. *c*, Each cone-like segment represents bounds on the estimate of curvature at a point. If such estimates were available, for example from the model that we propose, then the incorrect simple cell responses linking the two curves could be eliminated. A network for accomplishing this is described in detail in Parent and Zucker[15].

share the orientation preference of the excitatory centre[1,8]. Current evidence favours inhibition by a single large RF cell[9] for reversible inactivation of layer VI, which contains the long RF cells[10], eliminated or reduced endstopping in layer IV and the superficial layers; there is also anatomical evidence for a layer VI to IV connection[11,12].

With this background, observe that curvature can be defined as the rate of change of the tangent direction along a curve. For straight lines there is no change, whereas for circles the change is constant. Therefore, curvature varies directly with deviation from straightness over a local neighbourhood through which the curve passes.

In our model for estimating curvature, two simple cells estimate the straightness of a contour, one over a small neighbourhood and the other over a larger one, and the difference in their responses, which converge upon an endstopped simple cell (ES), varies with the deviation from straightness, that is, the curvature. Consider two similarly positioned and oriented simple cells with RFs of different size, termed the small and large simple cells. An endstopped simple cell receives excitation from the small cell and inhibition from the large cell, thereby encoding both orientation and curvature information. In symbols, let R_S denote the response of the small simple cell, R_L the response from the large simple cell to a particular contrast pattern at the same position and orientation, $\phi(\cdot)$ a rectifying or clipping function equal to its argument when positive (when excitation sufficiently exceeds inhibition) and zero otherwise, then the end-inhibited simple cell response is given by

$$R_{ES} = \phi(c_S \cdot \phi(R_S) - c_L \cdot \phi(R_L))$$

where c_S and c_L are positive constants that normalize the area difference between the receptive fields (Fig. 4). The non-linear clipping in the model increases the stimulus specificity by preventing a negative value for R_L from being flipped into a positive contribution to R_{ES}, the response of the ES cell. $\phi(\cdot)$ models the inability of spike trains to code negative numbers against a background of low spontaneous activity such as that found in area 17. The simple RFs can have either even or odd symmetry, that is, can be either symmetrical or anti-symmetrical about the central axis and Fig. 2 shows ES models composed of differences of each type.

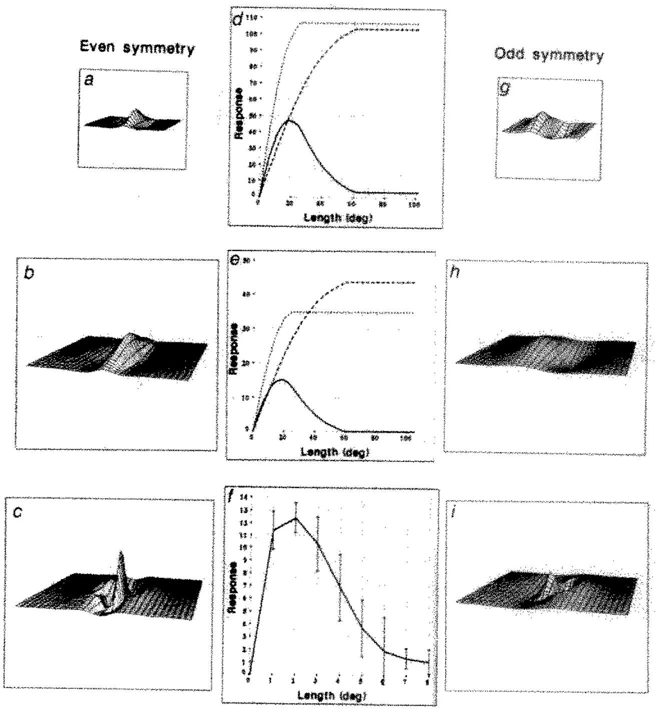

Fig. 2 The endstopped simple (ES) model. Binocular and temporal properties, and their non-linearities, are not incorporated, allowing the retino-geniculo-cortical pathway to be collapsed into a spatial receptive field, so the response of the cell can be modelled as a two-dimensional spatial convolution. Each simple RF may be modelled as a Gabor function or a difference of Gaussians (DOG). *a, b, c,* (Left column) Representation of the RF profiles of two even-symmetric simple cells and their linear combination. Each simple RF has a Gaussian weighting along the long axis and a DOG in the perpendicular direction. For these two simple RFs, the width ratio of the Gaussians comprising the DOG (σ_{x2}/σ_{x1}) is 2.5. The aspect ratios (σ_y/σ_{x1}) are 4 and 5 for the small and large RFs respectively. The smaller RF is 25 units long and the larger is 61. A linear combination of the two is also shown with weight constants $C_S = 3$ and $C_L = 1$. This graphic representation corresponds to the ES model only when the results of both simple convolutions are positive. (These parameter values are also used in Figure 3*d*.) *d, e, f* (Middle column), Length responses of even- and odd-symmetry ES models and of an end-stopped neuron respectively. *d*, The response of even-symmetry ES cell model to optimally-oriented bars of different length centered on the RF. The ES-cell model (solid curve) and the component responses of the two simple cells ($C_S \cdot \phi(R_S)$ and $C_L \cdot \phi(R_L)$, the dotted and dashed curves, respectively) were computed for bars 3 units wide with 50 lengths ranging from 2 to 100 units. *e*, The length-response of the odd-symmetry ES cell model (solid, dotted and dashed curves as in *d*). *f*, The actual response to moving bar stimuli of varying length obtained from an end-stopped neuron. *g, h, i* (Right column), Representation of the RF profiles of two odd-symmetry simple cells and their linear combination.

Methods. Surgery was performed under sodium pentothal anaesthesia with marcaine (a long-lasting local anaesthetic) applied around the surgical wounds. During the experiment, paralysis was maintained with continuous infusion of flaxedil (10 mg kg^{-1} h^{-1}) with anaesthesia by a respirated mixture of 70% N_2O/30% O_2 supplemented with intravenously infused nembutal (1 mg kg^{-1} h^{-1}). The animal was also infused with 5% dextrose in lactated Ringer solution (2 ml h^{-1}). Rectal temperature, end-tidal CO_2 and electrocardiogram were continuously monitored. Neuronal responses were assessed quantitatively by measuring: length tuning using optimally-oriented bar stimuli moving in the preferred direction, line-weighting functions (bars of the preferred orientation flashed throughout the central RF), and curvature-tuning with semi-circular arcs spanning a wide range of radii, moved in the preferred direction and positioned so that the tangent of the curve at mid-arc passes over the central RF parallel to the preferred orientation of the RF. For a more detailed description of our surgical and recording procedures see Ref. 13. *g, h, i,* The two odd-symmetry simple RFs are described by Gabor functions (a 2-D Gaussian multiplied by a sinusoid). For the small and large simple RFs (*g, h*), the aspect ratio (σ_y/σ_x) is 3 and the period ratio (λ/σ_x) is 2, where λ is the period of the sine function. *i*, Shows a linear combination of the two ($C_S = 2.5$ and $C_L = 1$). Again, the non-linear components of the model are omitted.

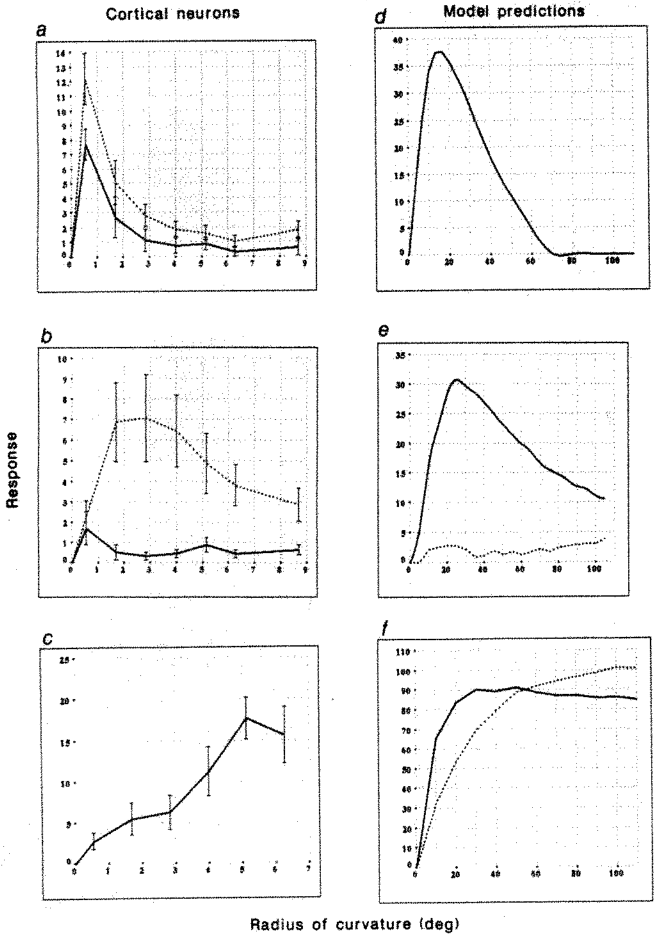

Fig. 3 Experimental (*d, e, f*) and theoretical (*a, b, c*) curvature-response data. Solid and dotted curves correspond to the two signs of curvature in all parts of the figure except *f*. *a, b, c* (Left column), Curvature-response of three area 17 neurons evaluated with semi-circular white arcs projected onto a darker screen. The tangent at mid-arc was parallel to the preferred orientation of the RF and moved in the preferred direction. The curves are based on sample means and standard errors from 16 randomly-interleaved presentations. A cubic spline was used to interpolate smoothly between the points in *a–e*. *a*, The curvature response of the endstopped neuron for which the length response is shown in Fig. 2*f*. The response does not vary with sign of curvature. *b*, Curvature response of an endstopped neuron. *c*, Curvature response of a nonendstopped neurone. *d–f* (Right column), Curvature response of even- and odd-symmetry instances of the model and the component responses of the even model produced by convolving the ES model with semi-circular arcs 3 units wide with different radii, to illustrate the sensitivity of the ES model to curvature. The curves are positioned so that the tangent to the curve at the point on the RF centre is parallel to the long axis of the RF. In each case the model was convolved with 20 curves varying in radius from 5 to 100 units. *d*, Curvature response of the even-symmetry ES model with the parameter values given in Fig. 2. *e*, Curvature response of the odd-symmetry model of Fig. 2 for the two signs of curvature, showing how different combinations of small and large cells give rise to different curvature selectivity and in particular, providing a suggestion for how different responses to the two signs of curvature could arise. *f*, Curvature response of the two simple components of the ES model in *d*, demonstrating how curvature selectivity arises. The non-endstopped neuron (*c*) and non-endstopped simulations (*f*) have a much broader band pass than the endstopped neurons and simulations.

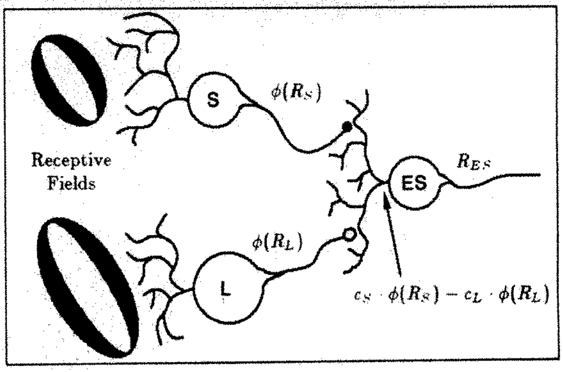

Fig. 4 A schematic model of the endstopped simple circuit. Neurons classified as simple cells with small (S) and large (L) receptive fields provide excitation and inhibition, respectively, to the endstopped simple cell (ES) upon which they converge.

Responses as a function of length for the even- and odd-symmetry ES models and their simple RF components are shown in Fig. 2*d, e*: a typical length response curve obtained in one of our neurophysiological experiments is shown for comparison (Fig. 2*f*). By varying the size of the two receptive fields, within physiological limits, and the weighting constants, it is possible to replicate any of the length-response data found in the literature while preserving position and orientation tuning. Although we shall not consider them further here, various combinations of weights and sizes are necessary to deal with the contrast and width variations that arise in natural images[14].

By analogy with a length-response curve, a curvature-response curve can be obtained by evaluating the model over a series of semi-circular arcs with increasing radius (Fig. 3). Two possibilities arise, one in which the model is composed of even-symmetry simple cells, and one in which they have odd symmetry. Consider the even-symmetry model first. For a stimulus with a low radius of curvature, the curve passes through the excitatory region of the receptive field of the small simple cell but does not pass through enough of the excitatory region of the receptive field of the large simple cell to inhibit it effectively. As the radius of curvature increases, the stimulus becomes increasingly effective in driving the large receptive field and the inhibition increases. Figure 3*d, f* shows the curvature response for the ES model and its even-symmetry components. For the odd-symmetry ES model, the curvature-response depends on the sign of curvature (Fig. 3*e*). For one sign of curvature, a curved stimulus excites the small RF but curves into the antagonistic region of the large RF, reducing its response and hence its inhibitory contribution to the ES cell. A similarly curved stimulus of the opposite sign passes through only the excitatory region of the large RF, allowing effective inhibition. For both signs of curvature, as the radius of curvature increases, inhibition becomes increasingly effective.

These computational results encouraged us to seek evidence for curvature-selective neurons in area 17 in the visual cortex of the cat. We will describe a cell as curvature-selective, if it responds strongly over only a limited range of curvatures when stimulated with a continuous arc falling within a range of positions and orientations at the centre of the RF. The curvature response of both endstopped and non-endstopped cells was examined to determine if curvature-selectivity is systematically related to endstopping. Cells were qualitatively characterized using a hand projector and then examined quantitatively using computer-controlled optics.

All of the fifteen endstopped neurons, both simple and complex, examined carefully so far exhibit the predicted peaked curvature response (Fig. 3*a, b*) whereas non-endstopped neurons exhibit a different, non-peaked response (Fig. 3*c*). The neuron of Fig. 3*a* displays similar curvature tuning for both signs of curvature, qualitatively resembling the even-symmetry model, whereas the one in Fig. 3*b* has virtually no response for one direction of curvature, similar to the odd-symmetry model. More than a third of the neurons exhibited significantly stronger responses to one direction of curvature than to the other, the cell in Fig. 3*b* being one of the most striking examples. Although most endstopped cells are directionally selective, we tested curvature response in both the preferred and null (opposite) directions of motion for some of the less directionally sensitive units. The results indicate similar curvature selectivity for both directions of motion.

In comparing the single unit results to the predictions of the ES model, note that layer VI contains complex RF types as well as simple, and they may also contribute to the generation of end inhibition and curvature-selective responses. Even so, an asymmetrical contribution from the large RF cell, whether of spatial or temporal origin, is required to generate responses selective for sign of curvature. Because experiments to determine the basis of this selectivity have not yet been performed, inhibition from a large, odd-symmetry inhibitory RF (Figs 2 and 3) must be considered as only one of the possible mechanisms.

Obtaining straightness (tangent) and curvature estimates from a difference of two first-stage responses has two advantages: anatomically, only vertical connections within a cortical column are required, and numerically, there is greater noise immunity in a first-difference operation than in repeated-difference operations.

Endstopping is usually thought to be related to length or end-point detection. We provide evidence for an alternative, that orientation-selective end inhibition allows the calculation of coarse-curvature estimates. Such estimates facilitate the process of orientation selection, and could be important in shape representation. As there are far more curves than end points in natural images, this proposal has a certain ecological plausibility.

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Kindling stimulation induces c-*fos* protein(s) in granule cells of the rat dentate gyrus

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Alterations in neuronal gene expression have been proposed to account for permanent changes in brain function such as learning and memory[1–4]. In particular, it has been suggested that proto-oncogenes such as c-*fos* (ref. 5) may be rapidly induced in conditions that lead to neuronal plasticity and evoke permanent changes in the expression of effector genes[1]. Concentrations of the c-*fos* proto-oncogene increase rapidly following depolarization-induced calcium influx in non-dividing neuronally differentiated PC 12 cells[2,3]. Recently, the presence and induction of c-*fos* in the adult brain and spinal cord has been observed[6–8]. Here we report that electrically-induced seizure activity, which leads to a permanent increase in the response of the brain to future seizures (kindling[9]), rapidly and transiently increases c-*fos* protein-like immunoreactivity in the nuclei of granule cells in the rat dentate gyrus. These results suggest that c-*fos* protein is present within the nuclei of adult mammalian neurons, and could be involved in plastic changes in the nervous system associated with seizure activity.

To study the effects of kindling stimulation on the production of c-*fos* protein(s), 40 adult male Sprague-Dawley rats were anaesthetized with barbiturate and bipolar stainless steel electrodes were implanted unilaterally into the dorsal hippocampus as previously described[10]. At least one week later, rats were electrically stimulated with high frequency trains (kindling stimuli: 500 μA, 100 Hz, 0.5 ms/half-wave diphasic pulses for 2 s) which produced brief partial hippocampal seizures, comprising an initial burst of high frequency and amplitude spiking lasting from 10–20 s, followed by a post-ictal electroencephalogram silence that was terminated by a second bout of afterdischarge lasting from 10–30 s. At various times after the spontaneous termination of these partial hippocampal seizures, rats were anaesthetized with barbiturate, and perfused and fixed for c-*fos* protein immunohistochemistry as previously described[6].

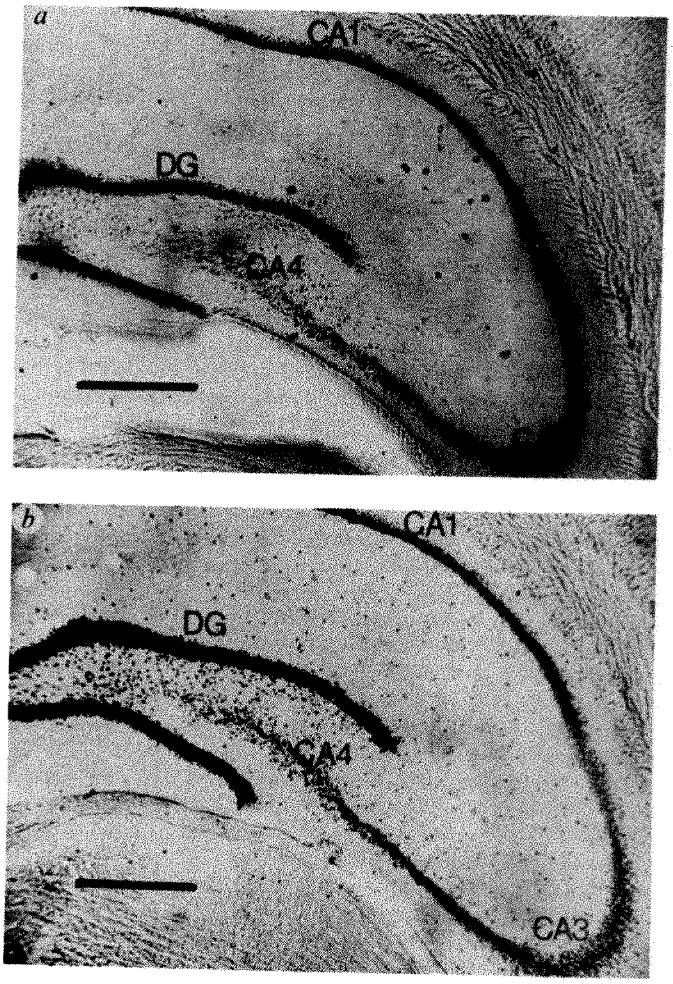

Fig. 1 *a*, Photomicrograph of rat hippocampal formation (areas CA1, CA3, and CA4) and dentate gyrus (DG) from an electrode implanted unstimulated rat. *b*, Photomicrograph of rat hippocampal formation and dentate gyrus fixed 30 min following a hippocampal seizure. Note the intense immunoreactivity in dentate granule cells and the increase in immunostaining in the pyramidal cells of areas CA1, CA3 and CA4. Antiserum was diluted 1 in 1,000 (Scale bar, 0.5 mm).

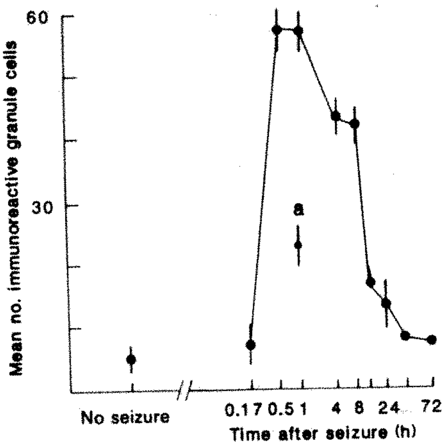

Fig. 2 Graph showing the average number of granule cells having positive c-*fos* protein immunoreactivity in an area of the dentate gyrus bounded by a 100 μm square. Values (mean ± standard deviation) were taken before, and at various times after, seizure activity at coordinates anterior/posterior −4.3 from bregma, using the atlas of Paxinos and Watson[17]. Point (a) denotes rats injected intraperitoneally with 40 mg per kg carbamazepine 30 min prior to seizure. Note the great reduction in immunostaining 1 h post-stimulation ($T = 3.4$, 9 d.f., $P < 0.05$).

Positive c-*fos* protein-like immunoreactivity was found within the nucleus of nerve cells (but not in glia) throughout the brain in a pattern similar to that found previously[6,7] (Fig. 1*a*). Neurons in the cerebral cortex, hippocampus, dentate gyrus, amygdala, caudate and piriform cortices showed positive immunostaining. Preadsorbing the antibody with the c-*fos* peptide[11] abolished the immunostaining. This result, together with the observed nuclear location of the immunostaining where c-*fos* proteins are known to accumulate[12,13], suggests that the immunostaining is to an endogenous c-*fos* protein-like molecule(s).

Rats that experienced seizure activity after electrical kindling stimulation showed a massive increase in c-*fos* protein-like immunoreactivity bilaterally in granule cells of the dentate gyrus (Fig. 1*b* and 2). This increase in immunostaining was rapid in onset, being present at 30 but not 10 min after seizure, and transient; the number of cells showing the response was almost back to baseline after 24 hours (Fig. 2). Immunostaining for c-*fos* protein was also increased in fields CA1, CA3 and CA4 of the hippocampus with a slower time-course (Fig. 1*b*), and in the piriform cortex. In rats that were electrically stimulated but treated with carbamazepine before stimulation to prevent seizures, a small increase in immunostaining, confined to the stimulated side, was observed one hour later (Fig. 2).

This seizure-related increase in c-*fos* protein immunoreactivity could arise from a number of possible factors, including the increased production of c-*fos* protein and interference with c-*fos* protein degradation. The latent period of onset of the effect corresponds to the onset of *de novo* synthesis of c-*fos* protein in culture systems stimulated by growth factors[14], suggesting that the increase is due to *de novo* synthesis of c-*fos* protein. This interpretation is supported by the work of Curran and his collaborators[7], who showed by Northern blot analysis that metrazol seizures increase the amount of c-*fos* messenger RNA.

These results demonstrate first that the c-*fos* protein(s) is present in the nuclei of adult rat neurons, which confirms previous results[6-8], and second, that activation of nerve cells with seizure activity producing kindling[10] greatly increases the production of the c-*fos* protein in granule cells of the dentate gyrus. The mechanism by which kindling-related seizure activity induces c-*fos* in granule cells is unclear. Calcium is known to accumulate in nerve cells during seizures[15], however, and induction of c-*fos* by nicotine and potassium in PC12 cells is calcium-dependent[2,3]. The molecular layer of the dentate gyrus has one of the highest densities of calcium antagonist binding sites in the rat brain[16] and this could account for the massive and rapid increase in c-*fos* in the dentate following seizures. The increase in c-*fos* is one of the earliest genetic events to follow neuronal activation[3]. It could fulfill a critical early function in coupling relatively brief electrical events to other long-term transcription-dependent events that maintain long-term changes in the nervous system, such as those observed in kindling.

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EDRF coordinates the behaviour of vascular resistance vessels

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Constriction of vascular smooth muscle in response to the stimulus of raised intravascular pressure—the myogenic response[1,2]—represents a positive feedback mechanism which, if unopposed, could theoretically lead to instability in the intact circulation[3,4]. Dilation in response to increased intraluminal flow would provide an opposing feedback mechanism which could confer overall stability[4]. Flow-dependent dilation in conduit vessels[5-7] is mediated by endothelium-derived relaxing factor (EDRF)[8-14], but the relationship between flow and EDRF activity has not been studied in resistance vessels *in situ*. We here demonstrate that EDRF can coordinate the aggregate hydrodynamic properties of an intact network. Under control conditions, EDRF maintains a fourth-power relationship between diameter and flow so that the pressure gradient in each vessel asymptotically approaches a constant value at high flow rates. Basal EDRF release may also maintain a similar spatial distribution of flow at different flow rates, even under conditions of moderate pharmacological constriction.

Vascular endothelium releases EDRF when stimulated by a variety of pharmacological agents[15]. The interaction of basally released EDRF[16-19] with myogenic responses in an intact vascular network might however be one of its major physiological functions. We have used a novel microangiographic technique[20] to investigate the influence of basal EDRF activity on diameter-flow relationships in an isolated, intact rabbit ear preparation (which has the advantage of being two-dimensional and relatively free of complicating metabolic influences) perfused under conditions of controlled, steady flow with physiological buffer. The method allows measurement of vessel calibre to the third generation of branch arteries (~70 μm in diameter). EDRF has

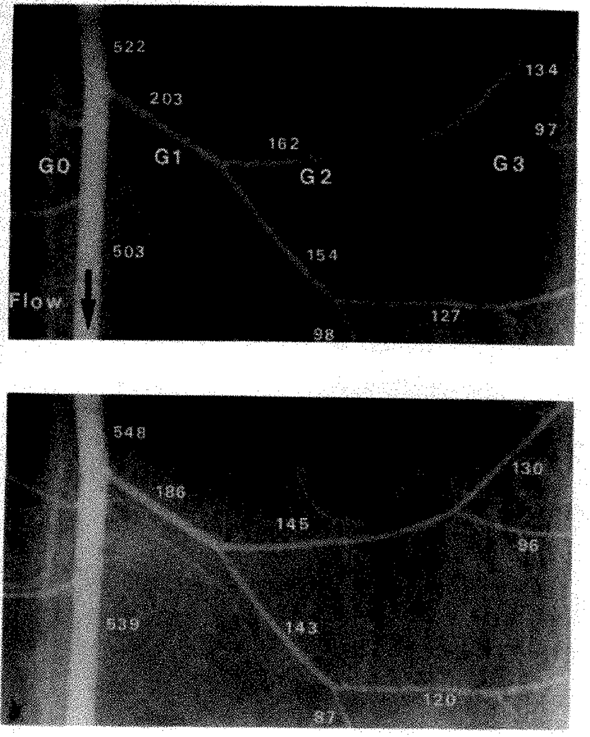

Fig. 1 Representative microangiograms of rabbit ear perfused at constant flow (2 ml min^{-1}) without (*a*) and with (*b*) 1 μM haemoglobin. Haemoglobin increased the pressure drop across the preparation from 35 mmHg to 50 mmHg, dilated the central ear artery (G0), and constricted the first, second and third generations of branch arteries (G1, G2 and G3). Vessel diameters (in μm) are shown.

Methods. Ears were removed from 2.5 kg male NZW rabbits killed by a blow to the neck and perfused by a Watson–Marlow pump with Holman's solution[17] (pO_2 450–550 mmHg at 35 °C). Phasic pressure variations were damped to <5% mean pressure by an air-filled compliance chamber. Zero pressure corresponded to zero flow. Experiments were conducted after 60–90 min stabilization. Microradiographs were obtained at steady-state perfusion pressure at each flow rate studied. A 4-μm microfocal X-ray source, exposures of 8–12 s and low kilovoltage techniques (10–25 kV) were used to optimize radiographic parameters. Radiographic contrast was provided by a pulse of low concentration non-ionic contrast medium (iohexol, 100 mg iodine ml^{-1}, diluted in Holman's buffer) which had negligible effect on perfusion pressure (<6% fall). The buffer contained 5% dextran (of relative molecular mass 80,000) to provide viscosity (2.23 mPas) equal to the contrast solution. Haemoglobin was freshly prepared by lysis of washed human red blood cells and chromatographic purification[24]. Measurement from microradiographs was assisted by an IBAS Kontron semi-interactive image analysis system.

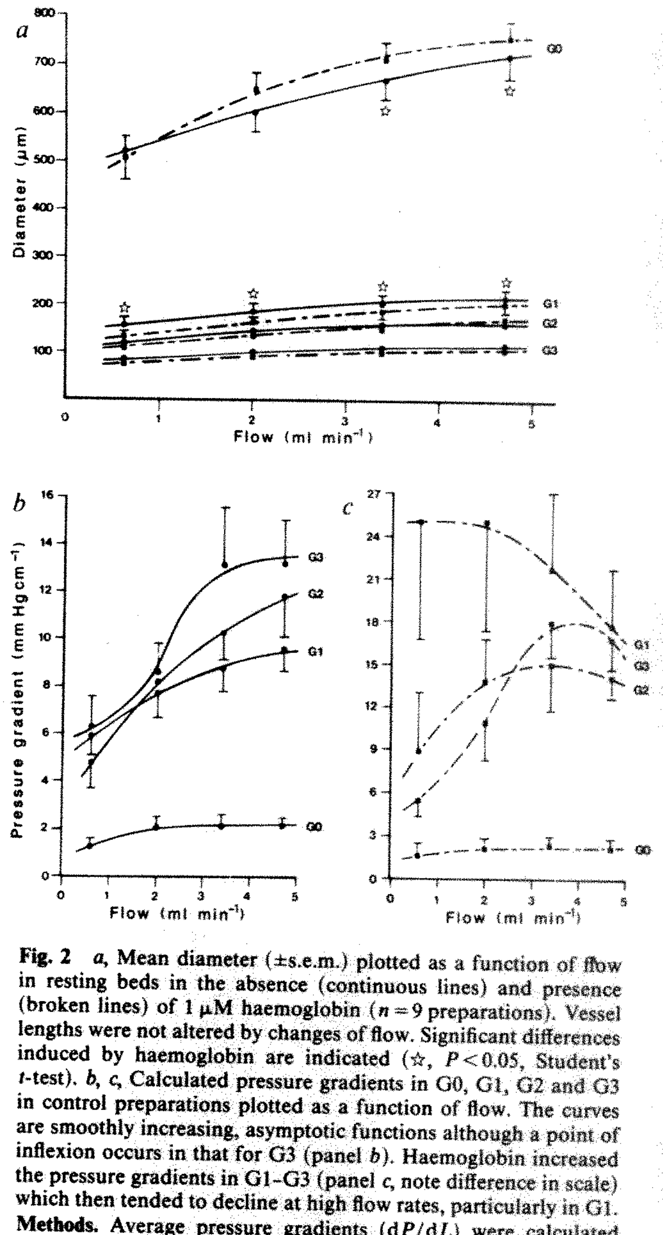

Fig. 2 *a*, Mean diameter (±s.e.m.) plotted as a function of flow in resting beds in the absence (continuous lines) and presence (broken lines) of 1 μM haemoglobin ($n = 9$ preparations). Vessel lengths were not altered by changes of flow. Significant differences induced by haemoglobin are indicated (☆, $P < 0.05$, Student's *t*-test). *b*, *c*, Calculated pressure gradients in G0, G1, G2 and G3 in control preparations plotted as a function of flow. The curves are smoothly increasing, asymptotic functions although a point of inflexion occurs in that for G3 (panel *b*). Haemoglobin increased the pressure gradients in G1–G3 (panel *c*, note difference in scale) which then tended to decline at high flow rates, particularly in G1.

Methods. Average pressure gradients (dP/dL) were calculated from the diameter–flow data illustrated in *a*, in the absence and presence of haemoglobin. In any generation $dP/dL = 128\mu/\pi NK \sum_{j=1}^{K} \dot{Q}/D_j^4$ where K is the number of preparations analysed (that is 9), μ is the dynamic viscosity of the buffer, D_j the diameter of the jth vessel and N the total number of vessels in each generation. N was estimated from the microradiographs as 1, 20, 75 and 250 in G0 to G3 respectively.

recently been shown to be nitric oxide[21], which is bound by haemoglobin[22]. In buffer-perfused preparations, haemoglobin inhibits EDRF activity induced by increased flow[12] and was used experimentally for this purpose; it does not increase vascular tone in the absence of endothelium[18,23,24].

In intact ear preparations, inhibition of basal EDRF activity significantly constricted the first generation of branch vessels (G1) at all flow rates, slightly constricted the second and third generations (G2 and G3) in some preparations, but generally dilated the central ear artery (G0) (Figs 1, 2*a*). The paradoxical dilation of G0 can be attributed to the overriding effect of a rise in perfusion pressure caused by 'downstream' constriction. These findings provide direct evidence that basal EDRF activity, by inhibiting spontaneous tone, influences the behaviour of resistance vessels *in situ*. They show that its influence is most evident in G1 in this preparation. It has been suggested that homeostatic responses to perturbations in pressure and flow within a vascular network will occur at sites where there is the greatest 'mechanical leverage'[25]. EDRF may function as just such a local regulator of flow: vessels in generation G1 divide without anastomosing (Fig. 1) and topographically are therefore 'feed vessels', well sited to control the distribution of flow.

Previous workers have assumed that a simple power law relates local values of diameter (D) and flow ($\dot{Q}$) in a microvascular bed, that is, $\dot{Q} = aD^M$ where a is a constant[26]. In the present study an expression of the form $\dot{Q} = aD^M + b$ was fitted to the diameter–flow relationships in each generation by a least-squares minimization procedure[27]: the constant b was inserted because vascular diameters are not zero at zero flow[28]. This expression was found to describe the aggregate behaviour of

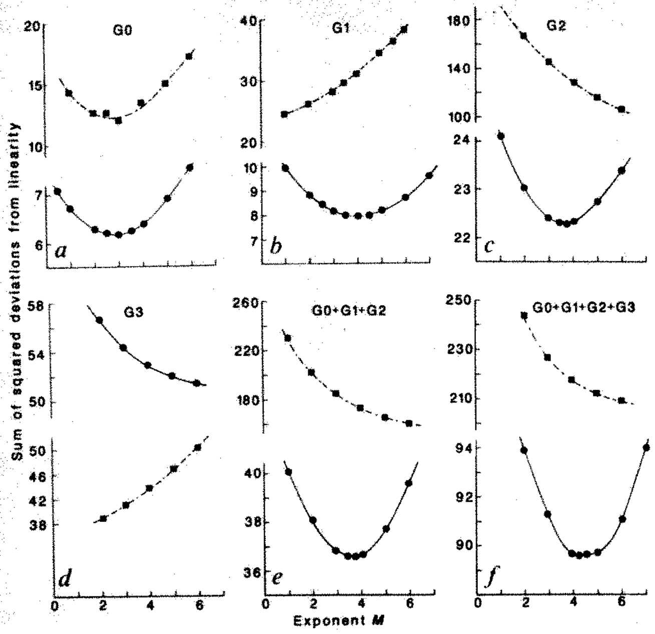

Fig. 3 Sums of squared deviations from linearity (SSD) after transformation of the diameter-flow data of Fig. 2*a*, according to the relationship $\dot{Q} = aD^M + b$, plotted against M in the absence and presence of haemoglobin. Using data for each generation ($n = 9$), minima were found in G0 (*a*), G1 (*b*) and G2 (*c*) but not in G3 (*d*), and also in pooled data G0-2 or G0-3 (*e*, *f*). In G0 haemoglobin did not abolish the minimum but increased SSD; in G1, G2, G0-2 and G0-3 haemoglobin abolished the minima. The M values of the minima in the absence of haemoglobin were G0 = 3, G1 = 4, G2 = 3.8, G0-2 = 3.75, G0-3 = 4.1. No minima could be found in the presence of 5-HT (either 0.1 or 1 μM, Fig. 4). Confidence limits for the estimates of M were sought by alternatively analysing data for individual vessels from the experiments of Fig. 2 and from the control data of Fig. 4 (total $N = 21$). Of all vessels, 55% exhibited minima. From this subpopulation estimates of M were 3.8 ± 0.6 in G0, 4.0 ± 0.3 in G1, 4.0 ± 0.4 in G2, 3.6 ± 0.4 in G3 and 3.9 ± 0.2 in G0-3 (mean ± s.e.m.).

Methods. Data were fitted by a microcomputer program to the equation $\dot{Q} = aD^M + b$. The computer chose a value of the exponent M and fitted a straight line through the four data points for each vessel by linear regression. These lines were then transformed to $Y = X$ where $Y = (\dot{Q} - b)/a$ and $X = D^M$, to normalize the data and render the analysis independent of vessel size and therefore branch generation. The sum of squared deviations (SSD) from linearity for the line $Y = X$ was then computed for each vessel, and hence the total SSD for any subgroup of arteries could be determined. If a valid linear relationship exists between $\dot{Q}$ and D^M, the total SSD for any group of arteries should exhibit a minimum when plotted against various values of M.

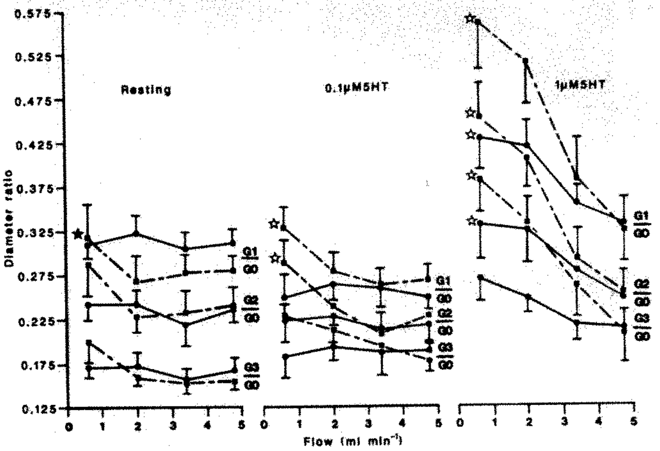

Fig. 4 The geometrical similarity of internal vessel diameters at each flow rate in generations G0 through G3 was compared by arbitrarily normalizing the diameters in G1, G2 and G3 as a fraction of G0 for each preparation. Data were obtained in unstimulated preparations (left, $n = 9$), and after pharmacological constriction with 5-hydroxytryptamine (5-HT) 0.1 μM (middle, $n = 5$), or 1 μM (right, $n = 7$), in the absence and presence of haemoglobin. Diameter ratios at each flow rate were compared non-parametrically (to avoid the assumption of their being normally distributed) by the Friedmann two-way analysis of variance by ranks technique[35], the null hypothesis being that they were independent of flow rate. The figure shows that this hypothesis was satisfied in resting and moderately constricted vessels but that the graphs tend to depart from the horizontal when EDRF is inhibited by haemoglobin and at higher levels of constriction (★, $0.1 > P > 0.05$; ☆, $P < 0.05$). If diameters were alternatively normalized with reference to G1, slopes deviated significantly ($P < 0.05$) from the horizontal in the presence of haemoglobin in G0 and G3 (but not G2) in resting vessels, and in G0, G2 and G3 in the presence of 0.1 μM 5-HT.

the network under control conditions (Fig. 3), but did not adequately fit data obtained in the presence of haemoglobin or during pharmacological constriction of the bed. The analysis assumes that the flow in any given vessel ($\dot{Q}$) is directly proportional to the input flow to the whole bed, as would be the case if its topography could be represented by a series-parallel network. This assumption is justified because anastomoses generally occurred only in G3 and higher order generations and because mean values of pressure and flow can be predicted from such a series-parallel model[29], even though branching patterns influence pressure-flow relationships[28-31]. In each preparation a mean 'spatially averaged' diameter for each of generations G1-G3 was obtained by meaning data from five separate vessels, and G0 was measured at a point midway between the base and tip of the ear. Figure 3 illustrates best estimates of the exponent M in different generations singly and in combination. In G0, where passive distensive effects may contribute, it was 3.0; in G1, where EDRF activity is maximal (Fig. 2), it was 4.0; in G2 it was 3.8; in G3 no minimum was found; pooled data from all generations gave a value of 4.1. These findings suggest that the influence of EDRF on flow-related vasomotion in these vessels may be described by a law of the form $\dot{Q} = aD^4 + b$ under normal control conditions. Although the experiments were conducted with non-pulsatile flow, it is unlikely that this conclusion would be different with pulsatile flow, which enhances EDRF release[11,12]. EDRF activity under these experimental conditions of steady flow appears to be maximal, in that 50 μM glyceryl trinitrate, which may be regarded as an EDRF analogue, did not reduce perfusion pressure in control preparations.

A fourth-power law has important implications. It implies that the pressure gradient in each vessel and thus the absolute pressure drop across it will become independent of flow at high flow rates: in Poiseuille flow the pressure gradient (dP/dL) is proportional to $\dot{Q}/D^4$, so that in vessels where $\dot{Q} = aD^4 + b$, dP/dL will be proportional to $a/(1 - b/\dot{Q})$ and asymptotically approach a constant value at high flows (see Fig. 2*b*). This finding is supported by experimental data showing near constancy of pressure gradient across the proximal vessels (down to 70 μm) of the cat mesentery subjected *in vivo* to changes in central artery pressure[30,31]. The general conclusion may thus be relatively independent of the complexities of pulsatile flow, dependency of blood viscosity on shear-rate and the spatial variation of haematocrit which occur *in vivo*[32]. A second implication of a fourth power law is that power losses become proportional to flow, since they are proportional to $dP/dL \times \dot{Q}$ in Poiseuille flow and dP/dL becomes constant at high flow. The energy expended in delivering a given volume of perfusate to the terminal elements of the vascular bed under control conditions will then become independent of flow rate, instead of being directly proportional to it as in a rigid tube. As the fourth-power law holds only in the presence of EDRF activity, EDRF appears to provide a mechanism which limits the work of perfusion.

EDRF also regulated the network in such a way that the relative change in diameter in each vessel was the same when flow was altered. Figure 4 illustrates this constancy of relative diameter, which was lost in the presence of haemoglobin and at high levels of constriction. Geometrical similarity of vessel diameters was maintained in partially constricted preparations and in G3; in neither of these was a fourth-power relationship between diameter and flow demonstrated, so it appears not to depend on this relationship. It implies geometrical similarity in respect of hydraulic resistance, which varies as L/D^4 and thus in practice on diameter alone (D) (as vessel lengths (L) remained constant). It follows that the spatial distribution of flow will be independent of flow rate. EDRF activity thus appears to interact with the myogenic and passive properties of the individual vessels, even in a partially constricted network, to coordinate the distribution of flow.

Murray[33] suggested that the optimum topography of a vascular network (under conditions of invariant flow) is one where flow is related to the cube of diameter because this minimizes power losses written as the sum of viscous power losses and a factor proportional to the volume of a section of artery ($W = a\dot{Q}^2/d^4 + bd^2$). Such a third-power law has indeed been shown to describe the regional dependence of flow on diameter in arterioles and arteries (6–108 μm in diameter) of the rat cremaster muscle[26], and to govern slow adaptation to altered flow in canine carotid artery[34]. Murray's analysis did not however take into account the possibility of flow-dependent dilation, nor did it address the effect of acute changes in flow—where we have shown that EDRF coordinates responses according to a fourth-power law. Whereas a third-power law minimizes power losses, a fourth-power law minimizes energy loss per unit volume which is proportional to $\dot{Q}/D^4$ in Poiseuille flow. EDRF could also provide the essential functional signal for the regulation of arterial calibre in the long-term, however, as a third-power law normalizes shear stress ($\propto \dot{Q}/D^3$) and EDRF activity is related to shear[11,12].

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Characterization of murine interleukin B by a monoclonal antibody

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Interleukin B (IL-B) (ref. 1), formerly termed BEF (B-cell-derived enhancing factor)[2,3] or IL-B4 (ref. 4), was originally described as a non-immunoglobulin regulatory factor spontaneously produced by B lymphocytes and B-cell lines that enhances the *in vitro* antigen-driven antibody response of unfractionated spleen cells stimulated by thymus-dependent antigens[2,3]. Since then we have examined the function of interleukin B in a number of immune reactions, both *in vitro* and *in vivo*[5], and found that it inhibits the activation of suppressor T lymphocytes[3,6–9]. We report here the production of two monoclonal antibodies (mAb) that specifically inhibit interleukin B activity. The use of these mAb in the purification and characterization of IL-B is described. IL-B from both normal and transformed B cells consists of two subunits of similar size and amino-acid composition. The structure of interleukin B and its specific behaviour in biological assay distinguish it from many other known lymphokines.

Murine IL-B was obtained from the non-immunoglobulin-expressing B-cell line P3-X63-Ag.8.653[10] and rat IL-B from the non-immunoglobulin-secreting B-cell line IR983F[11]. Partially purified IL-B of mouse and rat origin was used to immunize rats and mice respectively. Spleen cells from these animals were fused with the B-cell line P3-X63-Ag8.653. Hybridoma supernatant fluids were screened for anti-IL-B activity by solid-phase assay (ELISA) and supernatant from positive hybridoma clones was assayed for its capacity to inhibit IL-B enhancement of antibody synthesis by spleen cells[12]. Subcloning of selected anti-IL-B hybridomas produced two lines: MF-414.XIII (rat IgM) and MC-H23 (mouse IgG_1). Antibodies from both lines react in solid-phase assay (ELISA) with mouse and rat IL-B, and they were used interchangeably in the experiments dealing with murine IL-B. As illustrated in Fig. 1*a*, as little as 100 ng of purified MC-H23 antibody completely reversed the inhibitory activity exerted by IL-B on the activation of the suppressor T-cell hybrid T2D4-P. Comparable concentrations of an unrelated mouse mAb of the same isotype failed to inhibit the IL-B activity. Moreover, the same dose of MC-H23 inhibited IL-B enhancement of the antibody response (Fig. 1*b*). A similar inhibition of IL-B regulatory activities was found using purified MF-414.XIII antibody.

Both MC-H23 and MF-414.XIII mAb were used successfully partially to purify IL-B (see legend to Fig. 2). Purified biologically active IL-B was recognized by MC-H23 mAb in ELISA (Fig. 2, panel *A*). SDS-polyacrylamide gel electrophoresis (SDS-PAGE) analysis (14.5% polyacrylamide) showed that IL-B ran as two bands of relative molecular mass (M_r) 16,500 and 15,000 (16.5K, 15K). To test whether both proteins could be detected

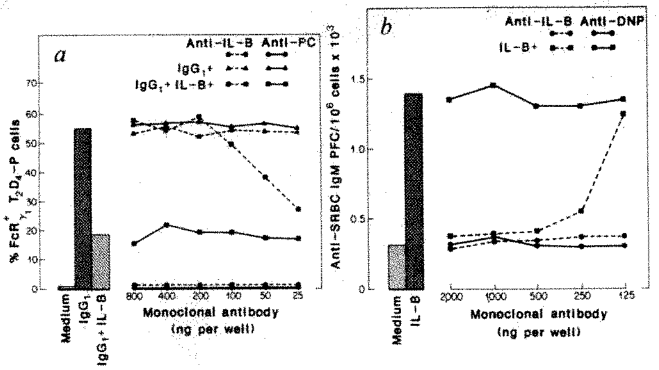

Fig. 1 Anti-IL-B mAb *a*, reverses the inhibitory activity exerted by IL-B on the activation of the suppressor T-cell hybrid T2D4-P, *b*, inhibits IL-B enhancement of antibody synthesis.

Methods. *a*, Lou rats and BALB/c mice were injected intraperitoneally with 10 μg (emulsified in 0.1 ml of complete Freund's adjuvant) partially purified mouse or rat (respectively) IL-B[12], and were boosted four times, at 4-week intervals, with 10 μg of IL-B adsorbed on 4 mg alum. Five days after the last injections, spleen cells were fused with the P3-X63-Ag8.653 line. Hybridoma supernatants (96-well plates) were screened for their capacity to react in a solid-phase assay with IL-B purified by affinity chromatography on an anti-IL-B rabbit IgG immunoadsorbent. Positive hybridomas were transferred into 24-well plates and supernatants (100 μl) from these were tested for their capacity to inhibit IL-B enhancement of *in vitro* antigen-driven IgM synthesis by spleen cells (10^6 cells per 0.1 ml)[12]. Two hybridomas that reproducibly inhibited IL-B activity were subcloned, resulting in two monoclonal antibodies: MF-414.XIII, which secretes rat IgM antibodies, and MC-H23, which secretes mouse IgG1. T2D4-P cells were generated by subcloning the suppressor T-cell hybrid T2D4, which can be activated by mouse IgG_1 to express $FcR\gamma_1$ (activation phase) and subsequently releases an IgG_1-specific suppressor factor (effector phase). It has been shown that IL-B is highly effective in inhibiting the activation of T2D4-P cells, but it is unable to inhibit the release of suppressor factor by already activated cells[5,7]. T2D4-P cells (5×10^5 cells in 0.1 ml) were cultured either in medium alone or in the presence of 20 μg murine IgG_1 per well (anti-DNP mAb 109.3) alone or together with 1.2 μg affinity-purified IL-B per well. Various concentrations of anti-IL-B mAb MC-H23 or anti-phosphorylcholine (PC) mAb 24B5, both of the mouse IgG_1 isotypes, were added to the culture. After a 2-h incubation the cells were assayed for $FcR\gamma_1^+$ cells by rosette formation with dinitrophenylated sheep red blood cells (DNP-SRBC) as described[9]. Similar results were obtained with the anti-IL-B mAb MF-414.XIII. *b*, Spleen cells from DBA/2 mice (10^6 in 0.2 ml) were cultured in the presence or absence of 2.4 μg affinity-purified IL-B per well and various concentrations of anti-IL-B mAb MC-H23 or anti-DNP mAb 109.3, both of the mouse IgG_1 isotype. Cultures were stimulated with SRBC, and direct anti-SRBC plaque-forming cells (PFC) were determined on day 5 of culture[6]. Similar results were obtained with the anti-IL-B mAb MF-414.XIII.

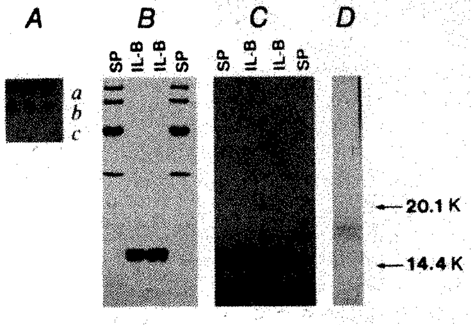

Fig. 2 Biochemical analysis of IL-B purified from a B-cell line or from normal B lymphocytes. Panel *A*, ELISA analysis of IL-B purified from a B-cell line. *a*, 15 ng or *b*, 7.5 ng RP-HPLC-purified IL-B or *c*, 15 ng mouse Ig were absorbed onto nitrocellulose paper (Millipore). The paper was blocked for 1 h at room temperature with 1% bovine serum albumin, washed with 50 mM Tris, *p*H 7.9, 150 mM NaCl (TBS) and then incubated for 2 h at room temperature with horseradish peroxidase-conjugated MC-H23 mAb in TBS containing 0.05% Tween 20. After washing, the paper was developed with a peroxidase substrate solution. This was prepared by mixing 2 ml 4-chloro-1-napthol (3 mg ml^{-1}) in methanol with 10 ml of TBS plus 0.01 M imidazole and then adding 30% hydrogen peroxide (5 μl). Panel *B*, SDS-PAGE (14.5% polyacrylamide) analysis of IL-B purified from a B-cell line. Electrophoresis of purified IL-B and molecular weight standard proteins (SP) was done under reducing conditions (2-mercaptoethanol), and proteins were detected by silver staining. Panel *C*, Western blotting of IL-B purified from a B-cell line. Electrophoresis as described in panel *B*; the protein bands were transferred to nitrocellulose paper using a trans-blot cell (Bio-Rad; 18 h at 10 mV) and visualized with horseradish peroxidase-conjugate MC-H23 mAb. Panel *D*, SDS-PAGE (14.5% polyacrylamide) of IL-B purified from normal B lymphocytes.

Methods. Serum-free culture supernatant fluids were applied to anti-IL-B mAb-coupled Sepharose columns and IL-B activity eluted with 0.2 M glycine, *p*H 2.4, buffer. Purified IL-B was subjected to reverse-phase high pressure liquid chromatography (RP-HPLC) on an aquapore octyl column (250×4.6 mm) (Brownlee, California) and eluted with a linear gradient of 40 to 60% acetonitrile over 30 mm at 2 ml per min. IL-B activity was eluted at 47-50% acetonitrile.

by monoclonal antibody, purified IL-B was electrophoresed and transferred to nitrocellulose paper. Figure 2 shows that MC-H23 mAb reacts with both 16.5K and 15K proteins but not with the proteins used as molecular weight markers (panel *c* and panel *b*). The amino-acid composition of the individual protein bands was determined after electroelution from the gel and was found to be closely similar. An amino-acid sequence could not be determined because the N-terminus of the proteins was blocked.

We then compared the size of IL-B produced by normal and unstimulated B lymphocytes with IL-B produced by a transformed cell line, B lymphocytes being purified as previously described[2] and cultured at a concentration of 10^7 cells per ml in medium containing 5% heated fetal calf serum (FCS). IL-B was purified by affinity chromatography and RP HPLC (see Fig. 2 legend) and chromatography fractions were screened in ELISA using the anti-ILB mAb MC-H23. Only one sample (eluted at approximately 50% acetonitrile) was positive. All samples were tested for their capacity to inhibit the activation of the suppressor T-cell hybrid T2D4-P, but only the sample positive in the ELISA showed inhibitory activity (55% inhibition). SDS-PAGE analysis (Fig. 2, panel *D*) showed a comparable M_r for active IL-B purified from normal and unstimulated B lymphocytes and IL-B purified from the continuous B-cell line. A biologically active IL-B purified from the B-cell line of rat origin IR983F[11] also interacts with the mAb MC-H23 and has a molecular size of 16K-18K (data not shown).

The specific activity of IL-B purified from the murine B-cell line was approximately 4,000 times higher than that of the crude supernatant fluid and 50 times higher than that of affinity-purified (MC-H23) IL-B. Figure 3*a* shows that 50 ng of IL-B purified by RP-HPLC reversed the activation of the suppressor T-cell hybrid T2D4-P. Figure 3 shows that 12.5 ng of IL-B resulted in a fivefold enhancement of the antigen-driven *in vitro* IgM antibody response.

Purified IL-B did not show IL-1, IL-2, BSF$_1$ (IL-4), BCGFII (IL-5) or interferon-γ (IFNγ) activity in specified biological assays. In particular, IL-B was tested for its capacity to increase the response of C3H/HeJ thymocytes to phytohaemagglutinin (IL-1), to sustain the growth of the T-cell line CTLL-2 (IL-2), to induce proliferation of NK cells (IL-2, BSF$_1$) or BCL$_1$ cells (BDGFII), to induce Ia expression on B cells (BSF$_1$) or on WEHI-3 cells (IFN$_\gamma$), and to activate macrophages for non-specific tumoricidal activity (IFN$_\gamma$) (P. del Guercio *et al.*, in preparation). Neither of the monoclonal antibodies described

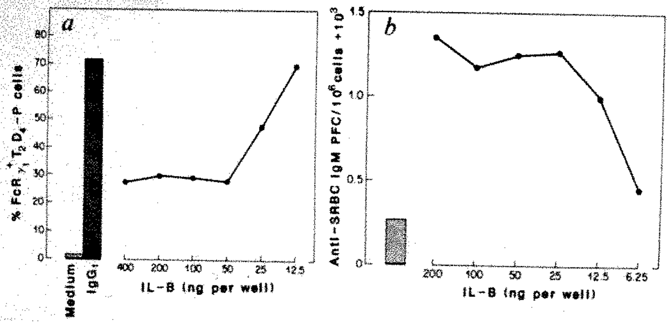

Fig. 3 RP-HPLC-purified IL-B *a*, inhibits activation of the suppressor T-cell hybrid T2D4-P; and *b*, enhances *in vitro* antibody response.

Methods. *a*, VT2D4-P cells (5×10^5 cells in 0.1 ml) were cultured either in medium alone or in the presence of 20 μg IgG_1 (anti-DNP mAb 109.3) per well and various concentrations of RP-HPLC-purified IL-B. After 2 h incubation the cells were assayed for $FcR\gamma_1^+$ cells by rosette formation. *b*, Spleen cells from DBA/2 mice (10^6 cells in 0.2 ml) were cultured in the presence of various concentrations of RP-HPLC-purified IL-B. Cultures were stimulated with SRBC, and numbers of direct anti-SRBC PFC were determined on day 5.

in this report reacts in ELISA with IL-1, IL-2 or interferon γ or with monoclonal mouse antibodies of various isotypes (results not shown). Moreover, on the basis of the amino-acid composition (Table 1), IL-B can be distinguished from other already sequenced lymphokines in that IL-B has no cysteine, a high content of alanine and lysine and a low content of leucine, aspartic acid and asparagine. These findings confirm earlier results[5] suggesting that IL-B is a unique lymphokine.

This characterization of a novel lymphokine is important for three reasons. First, these studies clearly establish the existence of non-immunoglobulin regulatory molecules produced spontaneously by B cells. Second, this is the first molecule to reduce the activation of suppressor T cells. In this regard, partially purified IL-B has been used successfully in mice to reduce the activation of suppressor T cells during acute graft-versus-host reactions (unpublished results). Third, IL-B could be important in the regulatory network that ensures the physiological dynamic equilibrium of the immune system. By inhibiting the activation of developing clones of suppressor T cells and therefore permitting the expansion of B-cell clones that are reacting against environmental epitopes, IL-B could promote the production of constant levels of immunoglobulin molecules in the absence of external stimuli[1]. The availability of an anti-IL-B monoclonal autoantibody, which is a powerful inhibitor of IL-B activity, will allow us and other interested groups to examine the role of IL-B *in vivo*.

Table 1 Amino-acid composition of mouse IL-B*

Amino acid	%
Aspartic acid/asparagine	6.2
Threonine	5.5
Serine	9.7
Glutamic acid/glutamine	9.0
Glycine	8.3
Alanine	9.0
Valine	6.9
Methionine	1.4
Isoleucine	4.1
Leucine	6.2
Tyrosine	4.1
Phenylalanine	6.2
Histidine	2.8
Lysine	11.7
Arginine	5.5
Proline	3.4
Cysteine	0

* Results are given for the 16.5 K protein. The 15 K protein has a similar composition.

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HLA-restricted recognition of viral antigens in HLA transgenic mice

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Cytotoxic T lymphocytes (CTL) recognize antigen in the context of the class-I products of the major histocompatibility complex (MHC)[1]. The extensive polymorphism of class-I molecules is thought to be linked to their capacity to present a large variety of foreign antigens[1]. Whether a single T-cell receptor (TCR) recognizes two separate epitopes (the foreign antigen and an epitope on MHC molecules[2,3]), or a single epitope resulting from the combination of a foreign antigen and an MHC molecule, has not yet been resolved[4-6]. In view of the differences between species in primary structure of histocompatibility antigens, it might be predicted that the TCR repertoire would evolve in concert with the diversity of MHC antigens. The mouse and human TCR repertoire would be optimally adapted to engage in productive interactions only with mouse (H-2) and human (HLA) MHC antigens respectively, especially if the more conserved features of histocompatibility antigens, in addition to foreign antigen, were seen by the TCR[7]. Alternatively, only the most variable segments of MHC antigens might be engaged in antigen presentation and thus in interaction with the TCR[8]. In that case, interaction between MHC plus antigen and the TCR might not necessarily be limited by species-specific features. By analysis of the T-cell response against virus-infected cells in HLA-B27/human β_2-microglobulin double transgenic mice, we report here that the mouse T-cell repertoire is perfectly capable of using the human HLA-B27 antigen as a restriction element.

The HLA-B27 specificity can be subdivided in a number of subtypes based on the reactivity of allospecific and HLA-

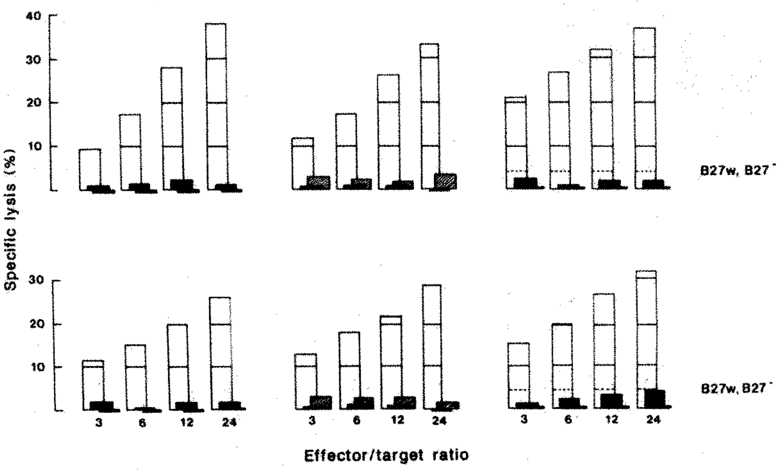

Fig. 1 Cytotoxic T lymphocytes (CTL) specific for Sendai virus (upper panels) and influenza virus (lower panels) were generated in double transgenic mice[16] and tested on autologous targets (left), H-2 matched non-transgenic targets (centre), and human HLA-B27K positive cells (right). In each case, CTL were tested on target cells infected with the virus against which the mouse was immunized (open bars), as well as on cells infected with the irrelevant virus (solid bars; influenza virus in the case of Sendai-specific CTL; Sendai virus in the case of influenza-specific CTL), and uninfected target cells (hatched bars), at the effector/target ratios indicated. Only those virus-infected targets expressing HLA-B27K or matched for autologous H-2 antigens are lysed. Lysis of human HLA-B27W$^+$ or HLA-B27$^-$ cells was below 4% specific release (indicated by the dashed line; rightmost panels) for virus-infected as well as uninfected cells (data not shown).

Methods. Double transgenic mice were primed *in vivo* by intraperitoneal injection of 1,000 haemagglutination units (HAU) of Sendai virus or 1,500 HAU of influenza virus A/X79 (FLU) in 0.5 ml of phosphate-buffered saline (PBS). Seven days after Sendai virus priming or 21 days after influenza virus priming, mice were killed and their spleen cells (5×10^7) stimulated with 10^7 virus-infected, irradiated (2,000 rad) autologous spleen cells in 45 ml of Iscove's modified Dulbecco's medium, supplemented with 10% (v/v) fetal calf serum (FCS), 100 IU ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and 5×10^{-5} M 2-mercaptoethanol. Autologous lymph node cells were cultured in the same medium and used as target cells. Stimulator cells were incubated with virus (300 HAU per 10^7 cells) in 1 ml of medium for 1 hour at 37 °C. Cells were washed twice and influenza-virus-infected stimulator cells were further incubated for three hours before irradiation. After five days, the responding CTL were collected and tested in the ^{51}Cr-release assay on Sendai-virus-coated (1 h) or influenza-virus-infected (18 h) target cells. Murine and human lymphocytes stimulated for three days with 2.5 μg ml^{-1} concanavaline A were used as target cells. Infection of target cells was performed as described for stimulator cells. Responding CTL were incubated with ^{51}Cr-labelled target cells at the effector/target ratio indicated. The percentage of specific release was calculated as (release in the presence of CTL − spontaneous release)/(release in 10% Triton X-100 − spontaneous release) × 100%. The spontaneous release in medium from labelled target cells ranged from 10% to 20% of the maximal release produced by Triton X-100. Human peripheral blood HLA-typed lymphocytes were isolated by flotation on Ficoll-Isopaque and stored in liquid nitrogen until used. Human cells were cultured in the presence of 10% normal human pooled serum. HLA types of the human cells were: HLA-A2, A28; B27K; Cw2; (B27K), HLA-A2, A28; B27W; Cw2; (B27W), and HLA-A2, A28, B5, B12; Cw4; (B27−). Bars, mean percentage of specific lysis of target cells at the given effector/target ratio measured in triplicate.

restricted, virus-specific CTL[9,10]. The genes encoding two distinct HLA-B27 subtypes (HLA-B27W and HLA-B27K), which together make up the vast majority of HLA-B27-positive individuals of caucasoid origin[10], have been cloned[11] and expressed in mouse and human cells by transfection[12]. The products encoded by these two genes differ by only three amino acids[11,13]. In the course of transfection experiments into murine recipient cells it became apparent that both the human β_2-microglobulin (β_2m) and the HLA-B27 gene are required to obtain a high surface expression of HLA-B27 antigens[12]. Only in such double transfectants, authentic HLA-B27 molecules, consisting of both human subunits, rather than hybrid products consisting of HLA-B27 and murine or bovine[12] β_2m are expressed at the cell surface. This situation is in contrast to HLA-A2 and HLA-B7 heavy chains, which readily associate with murine β_2m in transfection experiments[12,14,15]. We therefore constructed two sets of transgenic mice, one expressing the HLA-B27K gene, and the other the human β_2m gene. Crosses between mice of these two types produce animals expressing both human subunits at the cell surface and thus authentic HLA-B27 antigens, as verified by cytofluorimetry and biochemical analysis[16]. The amount of HLA-B27 antigen present in spleen cells from double transgenic mice is comparable to that found in Epstein-Barr virus (EBV)-transformed human lymphoid cell lines (unpublished observations). To confirm and extend these findings it was shown that human anti-HLA-B27K specific CTL[9] can efficiently lyse double transgenic murine target cells. Lysis of B27K positive double transgenic murine target cells was observed (29% specific release at effector/target ratio of 7:1), whereas control murine cells of HLA-B27-negative human cells were not lysed (data not shown).

HLA-B27 molecules have been characterized in humans as effective restriction elements for anti-viral CTL[9,10,17]. To examine whether HLA-B27 as expressed in double transgenic mice would function as a restriction element, mice were primed *in vivo* with either influenza virus A/X79 or Sendai virus, and the secondary virus-specific CTL response *in vitro* was measured by lysis of virus infected autologous, H-2 matched, or human cells. Figure 1 shows that antiviral CTL, obtained *in vitro* from double transgenic mice after priming *in vivo*, were able to kill virus-infected HLA-B27K-positive human target cells, whereas no lysis was observed of non-infected cells, or virus-infected cells expressing HLA-B27W, HLA-B5 or HLA-B12 antigens. A second set of mice were used for a similar experiment, shown in Fig. 2. The CTL populations obtained also lysed virus-infected H-2-matched target cells, derived from non-transgenic (CBA × C57Bl/6) F_1 animals. These mice possess the H-2^b and H-2^q haplotypes present in the strain combination in which the transgenic mice were generated[16]. Thus, in double transgenic mice both murine and human class-I antigens are used as restriction elements. The specificity of the CTL for the virus with which the mouse was immunized is apparent from their lack of reactivity with target cells infected with the irrelevant virus: anti-influenza CTL do not lyse Sendai-virus-infected target cells nor do anti-Sendau-virus CTL kill influenza infected targets.

Recognition of Sendai- and influenza-virus-infected cells was restricted by the HLA-B27 subtype in the context of which immunization had taken place. Hence, murine T-cells can distinguish the same HLA-B27 subtypes—differing by only three amino acids—as can human alloreactive and anti-viral CTL[9,10]. Note that this distinction is almost absolute. Thus, in both virus systems double transgenic mice were perfectly able to generate anti-viral HLA-B27-restricted CTL that specifically killed virus-infected HLA-B27-positive human cells.

A number of previous reports have dealt with recognition of HLA antigens by murine CTL[18-20]. Because these CTL were invariably raised in animals that had not acquired tolerance to xenoigeneic MHC antigens, H-2 restricted recognition of such 'foreign' antigens would be expected, and has indeed been observed[18-20]. Results from such studies reflect the ability of H-2-restricted T cells to distinguish several distinct epitopes on a non-self molecule. This is of course strictly comparable to the

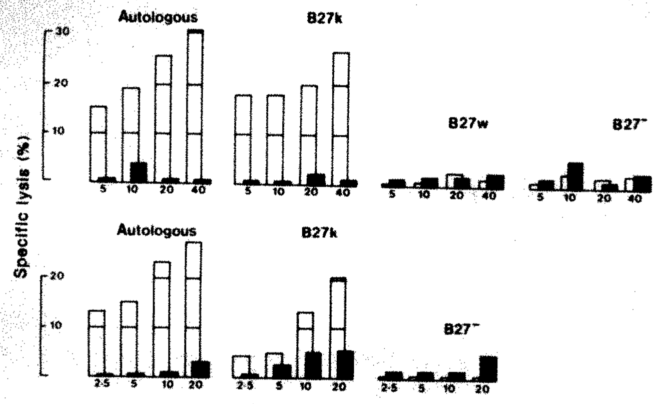

Fig. 2 Recognition of virally infected cells is restricted by the HLA-B27 subtype in the context of which was immunized. Immunization of mice, preparation of targets, and CTL assays were carried out as described in the legend to Fig. 1. In this experiment CTL were tested on infected (open bars) and uninfected (solid bars) targets only, having established that such CTL are virus-specific (Fig. 1). Bars indicate percentage of specific lysis of target cells by the given effector to target ratio measured in triplicate. The top panels depict the response of anti-influenza CTL on autologous, HLA-B27K-matched or mismatched targets, as indicated. The bottom panels show the results for the anti-Sendai response on autologous, HLA-B27K-matched, and HLA-B27 negative targets. The HLA-types of the targets were as described in the legend to Fig. 1.

ability of T cells to discriminate between epitopes on other 'foreign' antigens such as viral glycoproteins. In the experiments reported here, introduction into the germ line of all the information necessary to generate human HLA-B27 antigens has resulted in tolerance and has allowed us to test whether HLA-B27, next to the mouse's own class I MHC molecules, might serve as a restriction element for murine cytotoxic T cells. Our results show that this is indeed the case. It should be emphasized that authentic human HLA antigens are generated only when the genes encoding both human subunits are present, in contrast with the hybrid antigens produced if only HLA heavy-chain genes are used to construct transgenic animals[21]. In the latter case, a direct comparison of cells from transgenic mice with human cells as either targets or effectors should take into account the fact that human and mouse β_2m show only 70% similarity, and that hybrid molecules are distinct, at least serologically, from the parental molecules[22,23].

Our results strongly suggest that selection of the T-cell receptor repertoire is not appreciably affected by conserved regions of the MHC class-I molecule that contain 'species-specific' residues (for example the α-3 domain, or β_2m). The more conserved areas may merely serve to position the antigen-presenting segments of class-I antigens in the correct configuration in relation to the T-cell receptor. It is more likely that indeed the most variable regions of the class-I molecule are directly involved. Although some species-specific residues may be identified in these regions, they obviously still allow the selection of such T-cell clones that can use HLA-B27 as a restriction element.

In humans, several diseases show a strong association with the HLA-B27 antigen. At present, it cannot be decided whether this association is due to holes in the repertoire, resulting from the need for tolerance to HLA-B27 in HLA-B27 positive individuals, or alternatively, by unique properties of HLA-B27 as an antigen presenting element. The experiments reported here are a first step towards the validation of HLA-B27 transgenic mice as a possible tool in the study of HLA-disease associations.

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Cytotoxic T lymphocytes against a soluble protein

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Thymus-derived (T) lymphocytes recognize antigen in conjunction with surface glycoproteins encoded by major histocompatibility complex (MHC) genes[1–3]. Whereas fragments of soluble antigens are presented to T helper lymphocytes (T_H), which carry the CD4 antigen, in association with class II MHC molecules[4], CD8-bearing cytotoxic T lymphocytes (CTL) usually see cellular antigens (for instance virally-encoded proteins) in conjunction with MHC class I molecules[5,6]. The different modes of antigen presentation may result from separate intracellular transport: vesicles containing class II molecules are thought to fuse with those carrying endocytosed soluble proteins. Class I molecules, in contrast, can only pick up degradation products of intracellular proteins (see refs 7 and 8). This makes biological sense; during an attack of a virus, class I-restricted CTL destroy infected cells and class II-restricted T_H guide the humoural response to neutralize virus particles and toxins. But here we provide evidence that CTL specific for ovalbumin fragments can be induced with soluble protein, and that intracellular protein degradation provides epitopes recognized by these CTL. These findings suggest the existence of an antigen presenting cell that takes up soluble material and induces CTL.

It is well documented that fragments of cytoplasmic and membrane-associated proteins, such as influenza nucleoprotein and MHC molecules, can be seen in the context of class I molecules[5,9–11]. But the possibility that antigens taken up from outside cells can be presented to class I-restricted T lymphocytes

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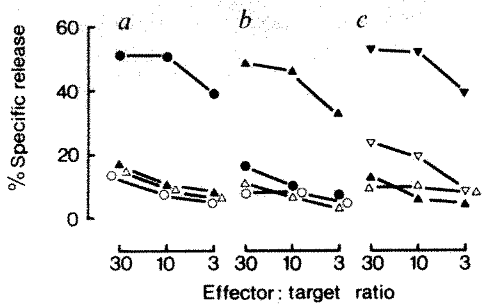

Fig. 1 Specificity of secondary *in vitro* cultures after *in vivo* immunization with soluble ovalbumin. Cultures derived from C57BL/6 (*a*, *c*) or DBA/2 (*b*) mice that had been immunized with ovalbumin in the presence (*a*, *b*) or absence (*c*) of syngeneic feeders were tested for their lytic activity on EL4 (C57BL/6, ●○▼▽) and P815 (DBA/2, ▲△) tumour targets in the presence (solid symbols) or absence (open symbols) of ovalbumin fragmented by trypsin digestion (*a*, *b*) or treated with CNBr (*c*).
Methods. Six-week-old C57BL/6 and DBA/2 (IFFA Credo) mice were intravenously injected either with a suspension of 2×10^6 syngeneic irradiated (2,000 rad) spleen cells in 0.5 ml of a solution of 4 mg ml^{-1} ovalbumin (Sigma) phosphate-buffered saline (PBS) after 30 min incubation on ice or with 0.5 ml 0.2 mg ml^{-1} ovalbumin in PBS. After 7 days animals were killed, spleens removed and cultured in single-cell suspensions in flat-bottomed 24-well plates (Costar) at 1×10^7 cells ml^{-1} in IMDM (Gibco) supplemented with 10% fetal calf serum (Boehringer), 2 mM glutamine, non-essential amino acids, 1 mM hydroxypyruvate, penicillin at 100 international units ml^{-1}, streptomycin at 100 μg ml^{-1}, gentamycin at 50 μg ml^{-1} (Sigma). Spleen cells obtained from animals immunized with feeders were grown in the presence of soluble ovalbumin at 0.7 mg ml^{-1}, to the other cultures CNBr-fragmented ovalbumin was added at 0.1 mg ml^{-1}. Ovalbumin was digested by trypsin (Sigma) as described previously[16] and fragmented by CNBr (Sigma) according to ref. 17 (tOva and cOva, respectively). Both preparations were checked for complete degradation by gel filtration. For the CML assay, ^{51}Cr-labelled target cells were preincubated with or without fragmented ovalbumin at 0.3 mg ml^{-1} in supplemented IMDM for 60 min at 37 °C. For the actual test 1×10^4 target cells suspended in the same medium were incubated for $4\frac{1}{2}$ h in round-bottomed microtitre plates (total volume, 0.2 ml; Costar) at effector to target ratios ranging from 30:1 to 1:1. Per cent specific lysis of these target cells was calculated as described elsewhere[5].

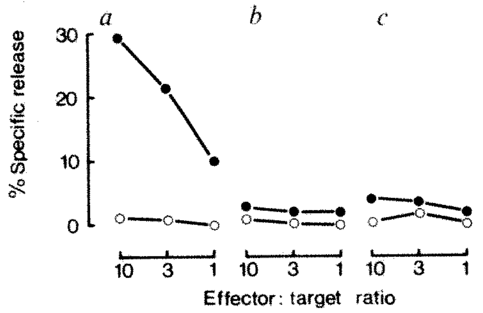

Fig. 2 Specificity of a cloned cytotoxic T lymphocyte line specific for CNBr-fragmented ovalbumin. The killing activity of C11 was tested against EL4 (*a*), P815 (*b*) and S.AKR (AKR/J, *c*) in the presence (●) and absence(○) of CNBr-fragmented ovalbumin.
Methods. The CTL clone C11 was isolated by limiting dilution from a secondary culture of spleen cells of a C57BL/6 mouse immunized with ovalbumin, and maintained by weekly subculture with 0.1 mg ml^{-1} CNBr-fragmented ovalbumin and irradiated syngeneic spleen cells in supplemented IMDM plus an exogenous source of IL-2. The CML assay was performed as for Fig. 1.

is suggested by several experimental lines of evidence. For instance, female mice reject syngeneic male skin-grafts after priming with male cells of another haplotype, and cytotoxic T lymphocytes of a given H-2 (mouse MHC) haplotype can be immunized against an array of minor and other antigens carried on cells of another haplotype[12,13]. Therefore, it was proposed that foreign antigens arising from the cellular debris are taken up by host cells and presented in conjunction with class I MHC[8].

We immunized mice with soluble ovalbumin, previously defined as a class II-restricted antigen. Irradiated syngeneic spleen cells incubated with a solution of ovalbumin were injected intravenously. Seven days later spleen cells were cultured in the presence of undigested ovalbumin for 5 days. Alternatively, soluble ovalbumin was only used for *in vivo* priming, and the spleen cells were grown *in vitro* together with CNBr-fragmented ovalbumin (cOva). To test for activity restricted by MHC class I molecules, tumour targets that do not express class II molecules were used (EL4, P815 and S.AKR). As demonstrated in Fig. 1, neither immunization protocol resulted in appreciably lytic activity towards allogeneic targets in the presence or absence of ovalbumin preparations. But C57BL/6 (H-2^b) and DBA/2 (H-2^d) mice immunized with ovalbumin mounted a specific response to the syngeneic targets, EL4 (H-2^b) or P815 (H-2^d) respectively, in the presence of their specific antigen, whereas in the absence of antigen only minor background activity could be observed. Preliminary experiments showed that in some cases minor but significant reactivity against ovalbumin could be obtained without prior *in vivo* priming (data not shown) suggesting that this 'background' reactivity might actually be directed against a component of the culture medium that can be presented by the target cell. The presence of syngeneic feeder cells at the time of immunization had no effect. Fragments of other proteins, for example pigeon cytochrome *c* or hen egg lysozyme (HEL), could not substitute for cOva or tOva. But CNBr-fragmented HEL could reveal specific MHC class I-restricted lytic activity after immunization with the soluble protein. Similar results could be obtained with blast cells as targets excluding any peculiar properties of the tumour cells (data not shown).

To further characterize the cell population responsible for this reactivity we established a cloned T-lymphocyte line, C11 (C57BL/6 derived) from a secondary *in vitro* culture directed against cOva. As determined by indirect surface immunofluorescence, C11 has the phenotype of a conventional CTL: CD1$^+$, CD4$^-$, CD8$^+$ (data not shown). C11 had a restriction pattern identical to the uncloned population (Fig. 2). The syngeneic target EL4 was susceptible to lysis only in the presence of cOva, otherwise it was not killed by C11, nor were allogeneic targets. These findings indicate that the induced MHC class I-restricted response can be carried by conventional CD8$^+$ CTL.

We wanted to examine whether this immune response against ovalbumin is directed against epitopes on this molecule that were expressed not only by chemical and enzymatic fragmentation, but also by intracellular protein degradation within the target cell. EL4 cells were transfected with BOVAneo expressing ovalbumin cDNA. The cell line EL4.Ova4-17 was subcloned and tested for susceptibility for lysis mediated by C11 (Fig. 3). The extent of lysis of the parental cell line in the presence of cOva (Fig. 3*a*) is comparable to that of the transfected cell line (Fig. 3*b*). In contrast, C11 could not kill EL4 grown in medium containing soluble undigested ovalbumin, excluding the possible explanation that secreted ovalbumin was taken up by EL4 and then presented in conjunction with MHC. It is noteworthy, however, that trypsin digestion of ovalbumin seems to destroy the epitope recognized by C11.

Although it cannot formally be excluded that ovalbumin is fragmented outside cells during the *in vivo* immunization, this explanation seems rather unlikely in view of findings that immunization with tOva, in all cases, failed to induce a measurable response (data not shown), whereas immunization with undigested ovalbumin always resulted in responses to epitopes

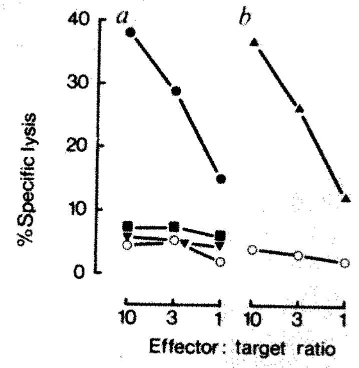

Fig. 3 Ovalbumin-transfected target cells can be lysed by the cloned CTL C11. The susceptibility to lysis of the parental line EL4 (○, ●, ■, ▼) in the presence of CNBr-fragmented (●), trypsin-digested (■) whole ovalbumin (▽) or uncoated (○) was compared to the ovalbumin transfected line EL4.Ova4-17 (△). **Methods.** Ovalbumin cDNA[18] was introduced into the BMIG*neo* expression vectors (H.K. & F. Melchers, in preparation) and EL4 cells were transfected with the resulting construct (BOVA *neo*). Neomycin-resistant EL4 clones were tested for ovalbumin expression by intracellular indirect immunofluorescence with a monoclonal mouse anti-ovalbumin antibody provided by D. Gray (data not shown). The line EL4.Ova4-17 was picked, subcloned by limiting dilution and tested for susceptibility for lysis mediated by C11. To test for presentation of undigested ovalbumin EL4 was grown in medium containing 10 mg ml^{-1} ovalbumin for 18 h.

exposed by trypsin digestion. As it is clear from our data that at least part of the activity induced by our immunization protocols is carried by conventional CTL recognizing epitopes found on ovalbumin, there cannot be a complete separation of the two pathways of antigen presentation for MHC class I-restricted and MHC class II-restricted antigens. Our data indicate the existence of a mechanism allowing exogenous antigens to be presented in conjunction with MHC class I molecules in a similar way to antigens expressed within the cell. The fact that EL4 grown in soluble ovalbumin cannot be lysed by C11, in contrast to its counterpart transfected with the ovalbumin gene, points to the existence of a specialized antigen-presenting cell responsible for the induction of CTL. But, in contrast to previous proposals[8], this antigen-presenting cell can take up soluble material. It should be of great interest to define the mechanisms and cell populations involved in the induction of CTL against soluble antigens further, as these cells might interfere with the immune response in the sense of a suppressive system.

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Loss of heterozygosity of chromosome 3p markers in small-cell lung cancer

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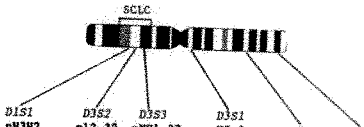
Specific chromosomal deletions sometimes associated with tumours such as retinoblastoma (chromosome 13q14)[1] and Wilm's tumour (chromosome 11p13)[2] have led to the hypothesis that recessive genes may be involved in tumorigenesis[3]. This hypothesis is supported by demonstration of allele loss specific for these regions using polymorphic DNA markers[4-9] and by the isolation of a complementary DNA clone for the retinoblastoma gene[10]. A cytogenetic deletion in chromosome 3 (p14-p23) was reported in small-cell lung cancer (SCLC) by Whang-Peng *et al*[11,12]. At least one homologue of chromosome 3 was affected in the majority of SCLC tumours; however, the multiple chromosomal changes seen presented the possibility that chromosome 3 was rearranged, not deleted. We used polymorphic DNA probes for chromosome 3p and compared tumour and constitutional genotypes of nine SCLC patients. Our data show loss of alleles of chromosome 3p markers in tumour DNA of all nine patients supporting the hypothesis that this region contributes to tumorigenesis in SCLC.

DNA samples prepared from tumour and normal tissues of the same individual were obtained from cultured cell lines H128

Table 1 Chromosome 3 markers in SCLC



	D1S1 pH3H2 HindIII	*D3S2* p12-32 MspI	*D3S3* pMS1-37 MspI	*D3S1* HS-3 HindIII	*CP* PstI	*SST* EcoRI
Group 1:						
H209BL	1,2	1,2	1,2	1,1	-	1,2
H209	1	1	1	1,1	-	1,2
N-1	1,2	1,1	1,1	1,2	1,2	1,1
SCLC-1	2	1,1	1,1	1,2	1,2	1,1
N-2	2,2	1,2	1,1	1,2	1,2	1,2
SCLC-2	2	2	1,1	1,2	1,2	1,2
N-3	1,2	1,1	1,2	1,2	1,2	1,1
SCLC-3	2	1	1	1,2	1,2	1,1
N-5	1,2	1,2	1,2	1,1	1,2	1,2
SCLC-5	2	1	1	1,1	1,2	1,2
N-6	2,2	1,2	1,2	1,2	1,1	1,2
SCLC-6	2	1	2	1,2	1,1	1,2
N-7	1,1	1,2	1,1	1,2	1,1	1,1
SCLC-7	1	2	1,1	1,2	1,1	1,1
Group 2:						
H128BL	2,2	1,2	1,2	1,2	-	1,1
H128	2	1	1	2	-	1
N-4	1,2	1,2	1,2	1,2	1,1	1,1
SCLC-4	1	2	1	1	1	1
Other lung cancers:						
N	2,2	1,2	1,1	1,2	2,2	1,1
Large cell	2,2	1,2	1,1	1,2	2,2	1,1
N	1,2	2,2	1,1	1,1	1,2	1,1
Adenocarcinoma	1,2	2,2	1,1	1,1	1,2	1,1

The numbers 1 and 2 refer to the alleles of a given locus. The copy number of homozygous markers is not known in the tumour samples. A dash indicates that the sample was not scored for a given polymorphism. Transferrin and somatostatin were not informative with *Eco*RI and *Bam*HI, respectively, for any of these individuals nor was pH3E4 for *Hin*dIII digests. Locus *D1S1* is now called *DNF15S1*.

and H209 with matching B-cell lines (H128BL and H209BL) and seven autopsy samples of normal and tumour tissue. All cases were histologically confirmed as small-cell lung cancer. Two non-small-cell lung cancers were examined which included a large-cell tumour and an adenocarcinoma of the lung. DNA samples were digested with the appropriate restriction endonucleases to detect DNA polymorphisms.

Eight probes detecting DNA polymorphisms localized to chromosome 3 were used to study the individual chromosome 3 homologues in SCLC tumours and normal tissue from each patient. Two probes lie within 3p14–p23, the region commonly deleted in SCLC as determined by karyotypic analysis. Probe pMS1-37 (detecting the locus *D3S3*) is localized to chromosome 3 (ref. 13) band p14.2 (ref. 14) and detects an *Msp*I polymorphism[15], whereas probe p12-32 (*D3S2*) is located at band p21 and has 2 alleles in *Msp*I digests[16]. The probe λH3(*D1S1, DNF15S1*)[17] has recently been reported to detect sequences on chromosome 3 as well as on chromosome 1 (refs. 18, 19), and the data of Donlon and Magenis[20] suggest that this marker localizes to 3p21. Two subclones of λH3(D1S1), pH3H2 (a 2-kilobase (kb) *Hin*dIII fragment) and pH3E4 (a 4-kb *Eco*RI fragment) were used to detect two independent *Hin*dIII polymorphisms which have been shown to be located on chromosome 3 (refs. 18, 19). The DNA markers on the long arm of chromosome 3 are HS-3 (*D3S1*) detecting a *Hin*dIII polymorphism at q12; transferrin (*TF*), an *Eco*RI polymorphism at q21; ceruloplasmin (*CP*) a *Pst*I polymorphism at q25; and somatostatin (*SST*) at q28 with polymorphisms detected with *Bam*HI and *Eco*RI.

The chromosome 3p markers *D3S2, D3S3* and *D1S1* were informative (that is, two different alleles were present in normal tissue) in all nine individuals, with six individuals heterozygous for two or more markers and three heterozygous for only one marker. Six individuals were heterozygous for *D3S3* alleles in DNA from normal tissue; all six had only one of the two alleles in the DNA from tumour tissue (see Fig. 1 and Table 1). Seven individuals were heterozygous for *D3S2* alleles in normal tissues, and had lost a *D3S2* allele in DNA from all of the corresponding tumours (see Fig. 1 and Table 1). All five individuals heterozygous for *D1S1* in normal tissue showed only one allele in the tumour. There does not appear to be a selective loss of a particular allele for any of the markers as either allele can be retained in different tumours. Tumour cell line H209, started from an untreated patient, had lost alleles of both *D3S3* and *D3S2* on chromosome 3p whereas markers on other chromosomes did not show allele loss.

Examination of polymorphisms over the length of chromosome 3 gave insight into the evolution of the chromosome 3 aberrations in SCLC. Table 1 lists the results for nine SCLC cases and two cases of large cell and adenocarcinoma of the lung examined with each of the chromosome 3 DNA probes. The samples fell into two groups: in the first and largest group

Table 2 Densitometry of DNA samples

			3p/3q				3p/3q		
		D3S3 4.8 kb allele / D3S1	T/N	D3S3 3.6 kb allele / D3S1	T/N	D3S2 2.9 kb allele / D3S1	T/N	D3S2 1.3 kb allele / D3S1	T/N
SCLC-2	Tumour	0		0.39	0.93	0	0	0.34	0.80
	Normal	0		0.42		0.55		0.43	
SCLC-3	Tumour	0	0	1.02	0.68	0.75	0.72	0	
	Normal	1.02		0.69		1.04		0	
SCLC-4	Tumour	0	0	0.54	0.14	0	0	0.08	0.20
	Normal	1.14		3.84		0.80		0.40	
SCLC-5	Tumour	0	0	0.64	0.29	0.45	0.48	0	0
	Normal	1.32		2.19		0.94		0.68	
SCLC-6	Tumour	0.95	1.31	0	0	0.87	1.19	0	0
	Normal	0.72		0.40		0.73		0.70	
Large cell	Tumour	0		1.86	1.08	0.67	0.62	0.90	1.45
	Normal	0		1.73		1.07		0.62	
Adeno	Tumour	0		2.24	0.88	0		2.56	1.41
	Normal	0		2.54		0		1.82	

			3p/15				3p/15		
		D3S3 4.8 kb allele / D15S1	T/N	D3S3 3.6 kb allele / D15S1	T/N	D3S2 2.9 kb allele / D15S1	T/N	D3S2 1.3 kb allele / D15S1	T/N
SCLC-2	Tumour	0		0.66	0.89	0	0	0.19	0.50
	Normal	0		0.74		0.50		0.38	
SCLC-3	Tumour	0.32	0.40	0.80	2.43	0.86	1.33	0	
	Normal	0.80		0.33		0.65		0	
SCLC-4	Tumour	0	0	0.88	0.58	0	0	0.22	1.00
	Normal	0.45		1.52		0.42		0.22	
SCLC-5	Tumour	0	0	0.65	0.47	0.80	0.84	0	0
	Normal	0.83		1.38		0.95		0.69	
SCLC-6	Tumour	1.92	1.31	0	0	3.32	1.68	0	0
	Normal	1.47		0.82		1.97		1.89	
Large cell	Tumour	0		1.90	0.70	1.49	0.55	2.02	1.30
	Normal	0		2.72		2.69		1.55	
Adeno	Tumour	0		2.71	1.18	0		4.01	0.77
	Normal	0		2.29		0		5.20	

To determine whether allele loss was associated with duplication of the remaining allele, scanning densitometry was performed on autoradiographs. To eliminate some of the variables in this method, the chromosome 3p data were normalized to probes on chromosome 3q and other chromosomes. Southern blots, which had been hybridized to chromosome 3p probes, were stripped of probe and hybridized to other probes. The 3q probe used was HS-3 (*D3S1*). The control chromosome used was pMS1-14 (*D15S1*). Similar results were obtained with p1E8 (*D13S4*) (data not shown). The autoradiographs were examined with a Hoeffer scanning densitometer (GS300).

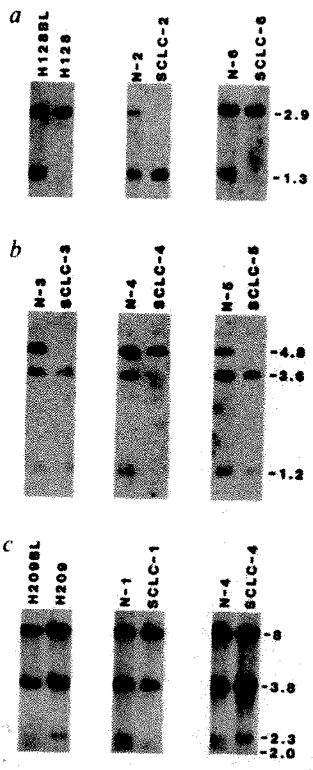


Fig. 1 Loss of alleles on chromosome 3p in small-cell lung cancer. Pairs of DNA samples from normal (left of each pair) and tumour (right) tissue from the same individual. *a*, *Msp*I digests hybridized to probe p12-32 (*D3S2*); allele 1 is 2.9 kb and allele 2 is 1.3 kb. *b*, *Msp*I digests hybridized to probe pMS1-37 (*D3S3*); allele 1 is 3.6+1.2 kb and allele 2 is 4.8 kb. *c*, *Hin*dIII digests hybridized to probe pH3H2; allele 1 is 2.3 kb and allele 2 is 2.0 kb. The 8 and 3.8 kb fragments are derived from both chromosomes 1 and 3. **Methods.** DNA from each sample (5 μg) was digested and hybridized as described previously[21]. Filters were washed and exposed to X-ray films at −70 °C using intensifying screens for 1-3 days.

of tumours, loss of alleles was detected only for markers on the short arm of chromosome 3. These data are consistent with an interstitial or terminal deletion in chromosome 3 or perhaps somatic recombination. The karyotype of tumour cell line H209 (ref. 11) demonstrates the presence of a terminal deletion of chromosome 3. Densitometry of X-ray films indicated that chromosome 3 was probably hemizygous for short-arm markers in SCLC-2, SCLC-3, SCLC-5 and SCLC-6 (Table 2). Because of the aneuploidy of these tumours, however, densitometry was not always conclusive.

Chromosomal nondisjunction, as well as somatic recombination, are postulated to have occurred in chromosome 13 in certain instances of retinoblastoma[4]. Group 2 consists of two sample pairs (H128 and SCLC-4) in which heterozygous markers seen in both the short and long arm of chromosome 3 in DNA from normal tissue are reduced to single alleles in DNA from the tumours. Densitometry of SCLC-4 (Table 2) suggests that chromosome 3 is hemizygous in the tumour. The karyotyped tumour cell line H128 has several copies of intact as well as deleted chromosomes 3 (ref. 11), all having the same set of alleles remaining for *D3S1*, *D3S2*, and *D3S3* (Table 1).

Because SCLC is highly aneuploid[11,12] we tested DNA markers on several other chromosomes (5, 8, 9, 10, 12, 13, 14, 15, 20, 21, 22). Some tumours showed loss of alleles on chromosomes 5, 13, 14, 15, 20 and 21. Tumours 1-6 were from patients who had received chemotherapy and four showed loss of an allele of one or more markers. Individual 7 had not received chemotherapy and all markers tested (other than chromosome 3) appeared to be the same in the tumour and in the normal tissue.

Karyotypic studies of SCLC using both cultured cell lines and direct tissue preparations have yielded variable results. Whang-Peng *et al.*[11,12] found the deletion in 3p in all 16 SCLC cases analysed whether or not the individual had been treated with chemotherapy. In contrast, Wurster-Hill *et al.*[21] found a chromosome 3 deletion in only 3 out of 15 cases studied. Other groups, however, have reported data similar to Whang-Peng: Yunis[22] found four cases of deletion in four SCLC tumours, Falor *et al.*[23] reported deletions in three of three tumours, Buys *et al.*[24] found deletions in three of three and Ibson *et al.*[31] eight of twelve tumours. Our studies using molecular probes demonstrate loss of chromosome 3 alleles at high frequency in SCLC (nine of nine cases). The two tumour cell lines and all seven of the SCLC samples obtained directly from the patient without intervening cell culture show loss of heterozygosity for 3p markers. Allele loss would not necessarily be observable karyotypically, perhaps accounting for the individuals which do not show a cytogenetic deletion in chromosome 3p in previous studies.

All karyotypic studies of SCLC have revealed a large number of chromosomal aberrations in the tumours[11,12,21-24]. Our studies with DNA markers for other chromosomes confirm the presence of a number of chromosomal changes in SCLC (data not shown). In contrast, studies with embryonal and childhood tumours[4-9] and acoustic neuroma[25] have shown little involvement of chromosomes other than the primary lesion. In adult bladder cancer Fearon *et al.*[26] also noted loss of alleles on a few chromosomes other than the commonly lost chromosome 11 markers.

The p21 region of chromosome 3 has been associated with at least two other types of adult cancer: a 3;8 translocation[27] and a 3;11 translocation[28] in renal cell carcinoma and chromosome 3p deletions and translocations in melanomas[29]. Hansen *et al.*[30] have given evidence that osteosarcomas that develop later in patients surviving retinoblastoma also involve loss of alleles on chromosome 13. These data imply that similar genetic loci may be involved in histologically dissimilar tumours. Whether the same chromosome 3 loci are involved in SCLC, renal cell carcinoma and melanoma remains to be determined. Analysis of DNA markers in these tumours will test the hypothesis that these tumours share a common chromosomal mechanism for tumorigenesis.

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Maternal inhibition of hepatitis B surface antigen gene expression in transgenic mice correlates with *de novo* methylation

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Differential modifications of the genome during gametogenesis result in a functional difference between the paternal and maternal genomes at the moment of fertilization$^{1-4}$. A possible cause of this imprinting is the methylation of DNA^{5,6}. The insertion of foreign DNA into transgenic mice allows the tagging of regions that are differentially methylated during gametogenesis. We describe here a transgenic mouse strain in which the expression of the hepatitis B surface antigen gene is irreversibly repressed following its passage through the female germ line. This inhibition is accompanied by the methylation of all the *Hpa*II and *Hha*I sites within the foreign gene, which we have shown to be integrated into a site on chromosome 13. The irreversibility reported here contrasts with what is found with other transgenic mice sequences which are reversibly methylated after passage through the male or female germ line^{7,8}, though in both cases methylation appears to be important in the imprinting process.

We have recently described two transgenic male mice containing hepatitis B virus (HBV) sequences in their genome and expressing hepatitis B surface antigen (HBsAg)^9. The sequences incorporated consisted of a 2.8-kilobase (kb) fragment of the HBV genome cloned into the *Bam*HI site of plasmid PBR322 (to give recombinant PAC2). Both mice, Tg(0HBV)E11-Pas (referred to as E11) and Tg(13HBV)E36-Pas (referred to as E36), contained one copy of the recombinant plasmid integrated close to the *Hin*dIII site used to linearize the plasmid (Fig. 1). The flanking sequences differed in the E11 and E36 strains, as judged by restriction mapping and hybridization with flanking sequences from the E36 strain.

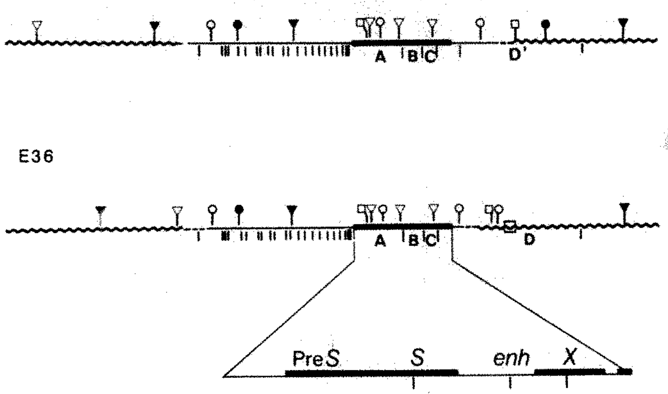

Fig. 1 Restriction map of the integrated PAC2 sequences in strains E11 and E36. A *Bgl*II HBV DNA fragment (solid line) was cloned into the *Bam*HI site of pBR322 (thin line). The HBV sequences are enlarged on the bottom line to show the genetic organization. Pre*S* and *S* encode the HBsAg polypeptides, *enh* is an enhancer region and *X* is an unidentified gene. Approximately one copy of the recombinant plasmid is inserted into the mouse genome (wavy line). A restriction map was made using *Eco*RI (sites shown as open circles), *Bam*HI (open triangle), *Bgl*II (open square), *Pst*I (dark circle) and *Pvu*II (dark triangle). The bars below represent *Hpa*II/*Msp*I sites. The E36 insertion was cloned into the λ vector L 47-1 and a probe (box) in the right flanking mouse DNA was isolated. It detected *Bcl*I and *Taq*I polymorphisms that segregated with markers for chromosome 13 (refs 10 and 11). For designation of A, B, C, D and D′, see text and Fig. 4.

Although the arrangement of the viral DNA was similar in both strains we observed two different types of transmission. The male mouse E11 transmitted the HBV-recombinant sequences to a male F$_1$ mouse produced by *in vitro* fertilization, then normally to next generation (N$_2$) males and females. In subsequent crosses, HBV DNA positive progeny always produced HBsAg irrespective of whether the plasmid was transmitted through the male or female N$_2$ mice (Fig. 2). The second mouse, E36, transmitted the HBV sequences without rearrangement to all its male and female F$_1$ progeny (designated H/+), and these always expressed HBsAg. HBV positive progeny from H/+ males in the second generation also always expressed HBsAg (Fig. 3*a*). Progeny of H/+ females receiving the HBV DNA did not, on the other hand, produce HBsAg and there was no transcription of the *S* gene in the liver. This was not due to gross rearrangement of the HBV sequences, as judged by restriction map analysis (data not shown). The mice were designated H^m/+ for modified or maternal HBV sequences. The loss of HBsAg expression was reproducibly observed when HBsAg-positive N$_2$ females were mated to normal males. The data show that female-transmitted HBV DNA sequences do not allow HBsAg expression in either the male or female progeny. To test the reversibility of this phenomenon, progeny from H^m/+ males and females were analysed for HBsAg expression. Among 17 HBV DNA-positive offspring from an H^m/+ male and 7 offspring from an H^m/+ female, none expressed HBsAg (Fig. 3*a*). This showed that the modification was not reversible even when the modified allele was transmitted by a male.

F$_1$ intercrosses were then set up to obtain homozygous offspring (Fig. 3*b*). The homozygotes received a modified allele of maternal origin and a normal one of paternal origin (H/H^m) which allowed HBsAg expression. HBsAg serum levels were similar in heterozygotes and homozygotes showing that neither H nor H^m was dominant. When crossed to normal mice H/H^m females produced 100% HBV positive, HBsAg negative progeny whereas H/H^m males gave 50% HBsAg positive animals and 50% HBsAg negative animals, confirming the irreversibility of the modification on passage through the male germ line. Homozygous H/H^m animals and their progeny developed normally, showing that insertion of the plasmid had not directly affected a region essential for either development or fertility. H^m/H^m homozygotes likewise developed normally and were fertile.

To test whether the lack of HBsAg gene expression in H^m/+ mice was due to a change in methylation status we carried out restriction enzyme digestions with *Msp*I and *Hpa*II. HBV sequences in the PAC2 plasmid contain three *Msp*I/*Hpa*II sites and pBR322 contains 22 *Msp*I/*Hpa*II sites (Fig. 1). Liver DNA from H/+ and H^m/+ mice was cleaved with *Msp*I and hybridized to an HBV probe (Fig. 4, lane 1). Three fragments of 1.3, 0.6 and 0.4 kb (bands A, B and C) produced by cleavage within the HBV DNA were detected, plus a 3.8 kb fragment (band D) corresponding to a site in the flanking DNA. A different pattern was obtained after digestion with *Hpa*II. In H/+ males and

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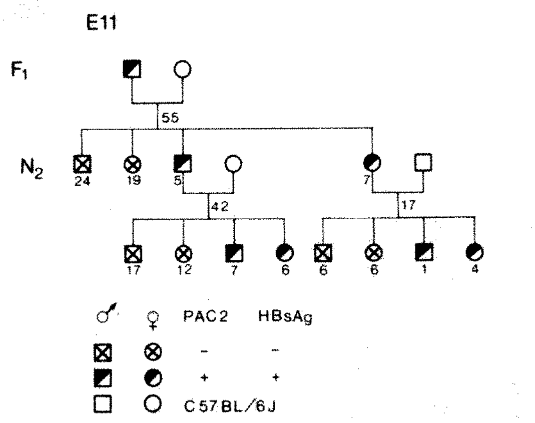

Fig. 2 Pedigree chart of strain E11. The founder male produced one offspring by *in vitro* fertilization. This mouse (male F_1) was crossed to C57BL/6-J-Pas females and produced male and female offspring, referred to as N_2. The mice were tested for HBV DNA using the dot-hybridization technique[9]. Serum HBsAg was measured with the AUSRIA radioimmunoassay (Abbott). The total number of offspring is indicated under each cross and the number of mice of each type indicated under the corresponding symbol.

females (Fig. 4, lanes 2 and 3), bands A, B and C were present without band D, showing that the first site in the cellular DNA was methylated. Additional bands corresponding to A+B and A+B+C arose from partial methylation of the HBV DNA. Hybridization with a pBR322 probe showed that the plasmid sequences were completely digested by *Hpa*II (data not shown). When liver DNA from male and female $H^m/+$ was analyzed in the same way the pattern obtained with *Hpa*II digestion was strikingly different (Fig. 4, lanes 4 and 5). Hybridization was found only in the high molecular weight DNA with both an HBV and a pBR322 probe, although complete digestion of the cellular DNA could be demonstrated by hybridization with a control probe. DNA from $H^m/+$ mice, but not from $H/+$ mice, was also resistant to *Hha*I digestion (data not shown). The same result was found with DNA extracted from several organs of a $H^m/+$ mouse, suggesting that the *de novo* methylation occurred in the early embryo or during oogenesis.

Liver DNA extracted from males and females of the E11 strain was also digested by *Msp*I (Fig. 4, lane 6) and *Hpa*II (Fig. 4, lanes 7 and 8) and hybridized to HBV. The A, B and C bands were detected in both *Msp*I and *Hpa*II digestions plus a 3 kb *Msp*I fragment (band D′) corresponding to a site in the flanking sequence. An additional band corresponding to A+B was also present.

The HBV sequences integrated in the E36 strain plus 2 kb of mouse flanking sequences were cloned, and a genomic probe devoid of repetitive DNA was isolated (Fig. 1). With this probe we have located the insertion site on chromosome 13 using an interspecific back cross between *Mus spretus* (SPE/Pas) and *Mus musculus domesticus* (C57BL/6-J-Pas)[10,11]. This region is methylated over a large distance in different tissues (including sperm) from adult male and female mice, in 11 day-old embryos, and in the F9 cell line (data not shown). Oocyte DNA was not tested.

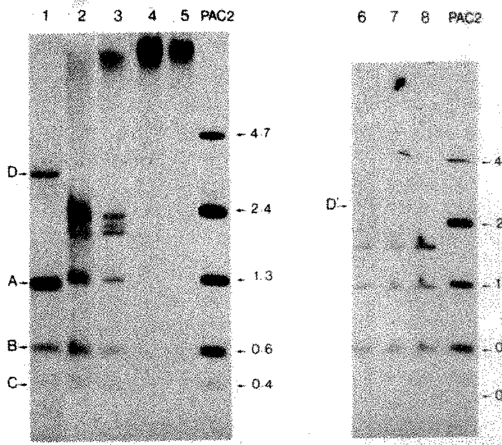

Fig. 4 Analysis of the methylation status of E11 and E36 liver DNA. DNA (15 μg) from the liver of males and females of the two strains were cut with *Msp*I or *Hpa*II and analyzed by electrophoresis on a 1.2% agarose gel and blot-hybridization[9]. Lanes 1 and 6: *Msp*I digestion of E36 and E11 liver DNA. Lanes 2 and 3: *Hpa*II digestion of male and female H/+E36 liver DNA. Lanes 4 and 5: *Hpa*II digestion of male and female $H^m/+$E36 liver DNA. Lanes 7 and 8: *Hpa*II digestion of male and female E11 liver DNA. The probe used to hybridize was the complete HBV DNA. PAC2 restricted with *Eco*RI or *Hpa*II (together in lane PAC2) served as a size marker. For designation of bands A, B, C, D and D′, see text and Fig. 1.

The *de novo* methylation of HBV sequences, on passage through the female germ line, is a consequence of its insertion into a particular site in the mouse genome and represents another example of a position effect[12]. It could reflect differential methylation of this region but this would necessitate its demethylation at some point during gametogenesis. One possible interpretation is that demethylation occurs in oocytes, followed by *de novo* methylation of the female allele only, in the early embryo. This could be another manifestation of imprinting, although different from that described by Reik *et al.*[7] and Sapienza *et al.*[8]. Chromosome 13 is not, however, thought to be involved in the parental effect because zygotes with maternal or paternal disomy survive normally[13,14].

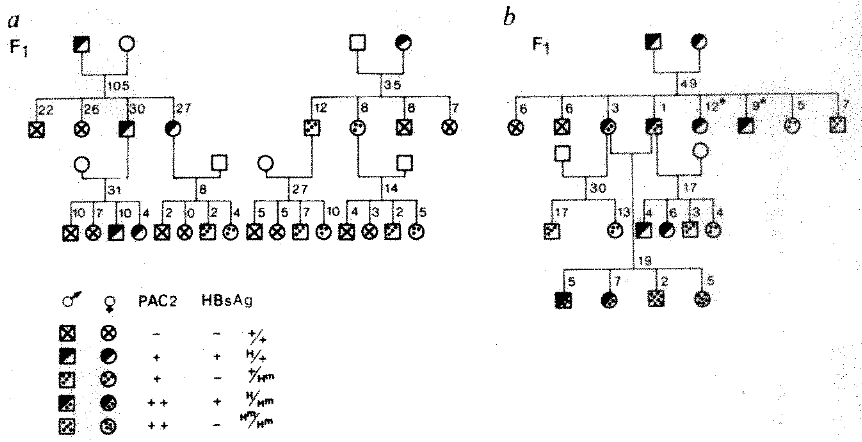

Fig. 3 Pedigree chart of strain E36. The founder male produced two types of offspring containing HBV DNA, only one of which produced HBsAg[9]. Mice of this latter type were selected for further studies. The first generation is referred to as F_1. Progeny were tested for the presence of HBV DNA and serum HBsAg as described in the legend to Fig. 2. *a*, Crosses between heterozygotes for the HBV insertion (referred to as H/+) and normal C57BL/6-J-Pas mice (open symbols). Three different F_1 males or F_1 females were crossed with normal mice. The modified HBV insertion transmitted by females is referred to as H^m. *b*, Crosses between two F_1 heterozygotes. The progeny of three different pairs of heterozygotes is reported. Homozygosity was first detected by hybridization and confirmed by pedigree analysis. * Total includes undetected homozygotes. H/H^m homozygotes were crossed to produce mice with two modified HBV insertions H^m/H^m which were fertile when crossed with normal mice (not shown).

Our findings also show that methylation of HBV sequences prevents *S* gene expression. Elsewhere we show (manuscript in preparation), that an *Hpa*II site in the *S* gene is specifically methylated in organs of H/+ mice that do not express HBsAg. This site is demethylated during liver differentiation. In the maternal inhibition described here, the extent of the methylation probably prevents reversibility. This can be compared with the irreversible inactivation of proviral sequences by methylation[15].

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p53 and DNA polymerase α compete for binding to SV40 T antigen

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The large T antigen (T) of simian virus 40 is a multifunctional protein required for both viral DNA replication and cellular transformation[1]. T antigen forms specific protein complexes with the host protein p53 in both virus-infected and transformed cells[2-4]. p53 has recently been shown to be an oncogene[5-7], but its normal function is not clear. We previously established a radioimmunoassay[8] to study the newly described complex between T antigen and DNA polymerase α, and have noted a similarity between the antigenic changes induced in T by the binding of both p53 and polymerase[9,10]. We now extend this analysis to a larger collection of anti-T antibodies and formally establish that p53 and DNA polymerase α can compete for binding to the SV40 T antigen. At a critical concentration of the three components it is possible to detect a trimeric complex of T, p53 and DNA polymerase α. Our observations have important implications for the control by these nuclear oncogenes of viral and cellular DNA synthesis and viral host range in both normal and transformed cells. We present a model for the action of p53 in growth control.

A quantitative radioimmunoassay for the complex between T antigen and DNA polymerase α was established by coating plastic microtitre wells with any one of a set of well-characterized anti-DNA polymerase α monoclonal antibodies[11]. These wells were then incubated with extracts of virus-infected cells and probed for the presence of DNA polymerase α-T complexes with a range of radiolabelled anti-T monoclonal antibodies. Control wells were coated with monoclonal antibodies of irrelevant specificity, and total T antigen in the extract was measured using an anti-T coated plate (Fig. 1*a*). Only a small fraction of the T antigen present in the extract appeared to be complexed to DNA polymerase α. The availability of large libraries of monoclonal antibodies to T, recognizing discrete epitopes on the protein, allowed an examination of the epitopes displayed by T bound to DNA polymerase α. Some of the anti-T monoclonal antibodies, for example PAb 250 (binding site between amino acids 271 and 367 of T) and PAb 204 (binding site between amino acids 453 and 469 of T), could not detect the T present in complex with DNA polymerase α (Fig. 1*b*). These same antibodies are unable to detect T that is complexed to p53 (ref. 10). It appeared therefore that DNA polymerase α and p53 could be occupying similar sites on T antigen or inducing the same conformational changes in the protein.

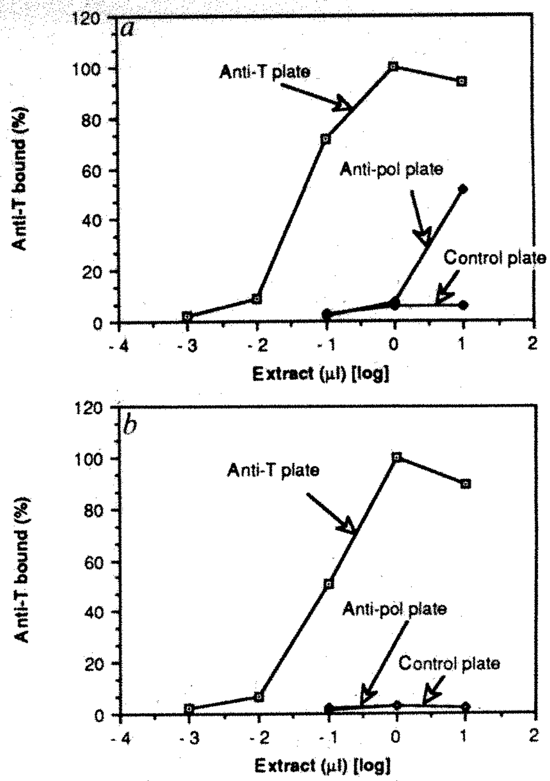

Fig. 1 Radioimmunoassay of the DNA polymerase α-T complex. T antigen and the DNA polymerase α-T complex present in an extract of Ad-5 SVR 111-infected 293 cells were estimated using a monoclonal antibody sandwich immunoassay with labelled anti-T antibodies and anti-T (PAb423, ref. 18) anti-DNA polymerase α, (SJK 287-32, ref. 11), or control monoclonal solid phase antibodies. The anti-T label is *a*, PAb 419 (ref. 18) and *b*, PAb 250 (ref. 13.) (A similar result to that shown in *b* was obtained using PAb 204 as label). Binding of each anti-T label is expressed as % of the maximum binding achieved for comparison between antibodies[9,10].

Methods. The cell extract was prepared from 293 cells grown on monolayers and infected with virus at a m.o.i. of 10 p.f.u. 24 h earlier[14]. The extract was prepared as an S100 (ref. 13) in 10 mM HEPES, *p*H 7.4, containing 1.5 mM $MgCl_2$ and 5 mM KCl (HYPO buffer) at a ratio of 1.5×10^8 cells ml^{-1}. All monoclonal antibodies were purified by protein A-Sepharose chromatography. For the solid phase, antibodies were used at a concentration of 30 $\mu g\ ml^{-1}$ to coat the wells of 96 well plastic microtitre plates. After coating overnight, plates were blocked by overnight incubation in a 20% solution of dried skimmed milk in PBS and then rinsed in PBS. Extracts were incubated on the antibody-coated plates overnight at 4 °C and, after rinsing in PBS containing 0.1% NP40, probed with the appropriate iodinated antibody for 4 h before washing and counting the individual wells. All assay points were determined in duplicate. Antibodies were labelled with ^{125}I to specific activities of 5 $\mu Ci\ \mu g^{-1}$.

To test this idea directly we established an *in vitro* assay for the assembly of the DNA polymerase α-T complex on the same principle as one used earlier for the T-p53 complex assembly[10]. The assay is capable of measuring the assembly of the T-DNA polymerase α complex, and both components of the complex can be independently titrated; a titration for T antigen is shown (Fig. 2). Maximum binding of the DNA polymerase α present

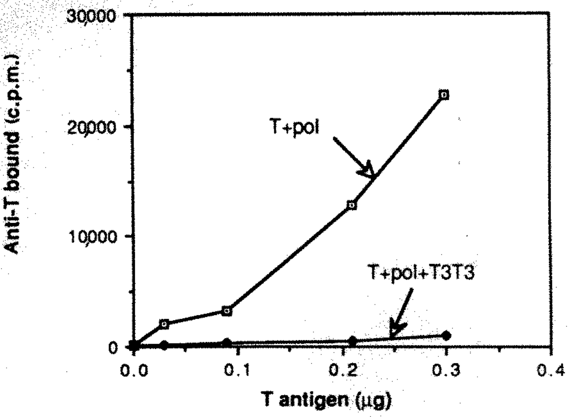

Fig. 2 Extracts of murine T3T3 cells block the *in vitro* binding of T antigen to DNA polymerase α. T antigen binding to DNA polymerase α *in vitro* was measured using an SJK 287-38 anti-DNA polymerase α solid phase and an iodinated anti-T, PAb 419, as a label. 50 µl HeLa S100, a source of DNA polymerase α, was mixed with 50 µl extract (10^8 cells ml^{-1} in 50 mM Tris, *p*H 8.0, containing 5 mM EDTA, 2 mM PMSF and 150 mM NaCl) of T3T3 cells, 'T+pol+T3T3', or 50 µl buffer alone, 'T+pol'. Variable amounts of pure T antigen in 10 µl buffer (20 mM HEPES, *p*H 7.4, 0.1 mM EDTA, 5 mM NaCl, 10% glycerol, 1% BSA) were added to the mix. After incubation for 2 h at 4 °C the mixtures were transferred to the solid phase antibody plate and incubated, washed, and probed with labelled antibody as described in Fig. 1.

in 50 µl HeLa cell S100 extract[12,13] is seen with 0.5 µg of immunoaffinity purified T antigen[14]. From the conditions of the assay we can calculate that the minimum affinity constant of T for DNA polymerase α must be at least 10^6 M^{-1} and could greatly exceed that figure if only a subfraction of T is competent to bind DNA polymerase α. The subunit composition of the mammalian DNA polymerase α remains controversial[15] and it is not known whether T interacts directly with the catalytic core polypeptide (against which the anti-DNA polymerase α antibodies used here are directed[11]), or with one of the associated polypeptides. Indeed it is possible that T itself may alter the subunit composition of the holoenzyme, perhaps substituting for a cellular subunit. To ask whether p53 could inhibit the binding of DNA polymerase α to T, a competition experiment was set up in the *in vitro* assembly assay. An S100 extract of HeLa cells served as a source of DNA polymerase α and an extract of murine T3T3 cells (a line of spontaneously transformed 3T3 cells[10] containing high levels of p53) was used as a source of p53; the two extracts were mixed and incubated with a range of T antigen concentrations. The T3T3 extract clearly inhibited the *in vitro* binding of DNA polymerase α to T (Fig. 2). To determine if p53 was the active component in the T3T3 extract responsible for this inhibition, the extract was selectively depleted of p53 by immunoabsorption on an anti-p53 monoclonal antibody column. A p53 radioimmunoassay confirmed that the extract was completely depleted of p53 (Fig. 3*a*). This depleted extract was unable to block the association of T and DNA polymerase α (Fig. 3*b*), confirming our hypothesis that p53 is the component responsible for the inhibition. Passing the T3T3 extract through control columns of anti-polymerase or anti-T antibodies did not remove p53 or deplete the extract of its inhibitory activity, though we could show these columns to be effective.

T antigen is found in infected and transformed cells in a variety of oligomeric forms[1]. In particular the T-p53 complex is highly oligomeric, containing multiple T and p53 subunits[1,3,4]. T purified by immunoaffinity chromatography[14] is at least tetrameric, so that at subsaturating levels of p53 and DNA polymerase α it should be possible to assemble a complex of p53 and DNA polymerase α 'bridged' by T antigen. To identify such a complex, a variable amount of T3T3 extract was mixed with pure T antigen after mixing with control buffer or with HeLa cell extract (Fig. 4). Using appropriate immunoassays, the formation of T-pol complex, (Fig. 4*a*), T-p53 complex (Fig. 4*b*), and the postulated pol-T-p53 complex (Fig. 4*c*) was measured. The results show (Fig. 4*a*) that detection of T-pol complex is, under these conditions, absolutely dependent on the presence of the HeLa extract and that the formation of this complex is suppressed by high concentrations of murine p53 (from the T3T3 extract). The formation of the T-p53 complex (Fig. 4*b*) is not dependent on, or suppressed by, the HeLa extract, but is dependent on the T3T3 extract. Finally, a trimeric complex of pol-T-p53 can be detected, using an anti-pol solid phase and an anti-p53 label (Fig. 4*c*). The formation of this complex reaches a sharp peak within the narrow T3T3 extract (p53) titration range and high concentrations of p53 inhibit the formation of the complex since all binding sites on the T oligomer are occupied by p53. The formation of this p53-DNA polymerase α complex only occurs in the presence of T antigen.

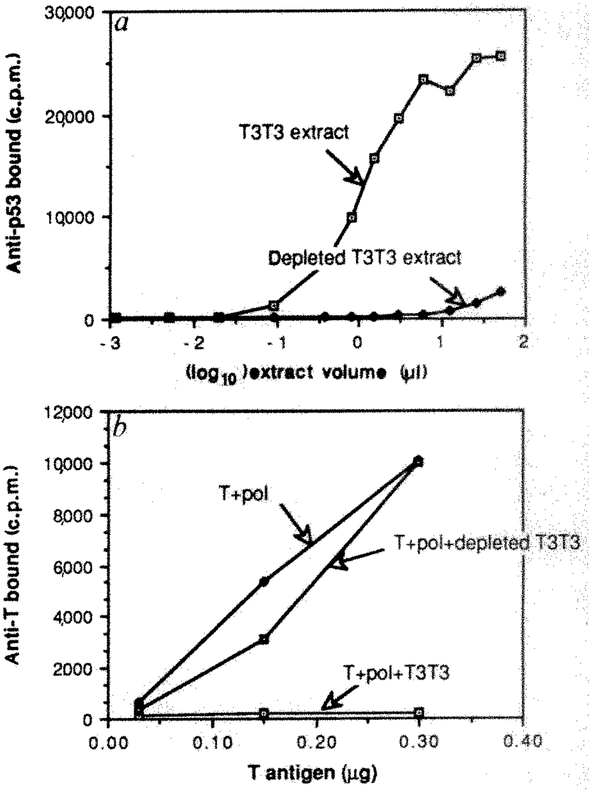

Fig. 3 Murine p53 blocks T antigen binding to DNA polymerase α. *a*, Immunoaffinity depletion of p53 from the T3T3 extract. An extract of T3T3 cells prepared as described in Fig. 2 was depleted of p53 by incubation with the anti-p53 antibody PAb 421 (ref. 18) covalently coupled to protein A-Sepharose beads[14]. The effectiveness of the depletion was monitored by titrating the starting extract and the depleted extract in a p53 sandwich immunoassay[19,20]. This assay used the anti-p53 RA3 2C2 (ref. 21) as a solid phase and another anti-p53 200.47 (ref. 22) as the labelled probe. Wash conditions and incubation times are described in Fig. 1. *b*, T3T3 extracts depleted of p53 no longer inhibit the binding of T to DNA polymerase α. The assay was run as described in Fig. 2 except that, in the case of 'T+pol+depleted T3T3', the total T3T3 extract was replaced with 50 µl T3T3 extract, depleted of p53 by immunoaffinity chromatography as shown in Fig. 3*a*.

The finding that the cellular protein p53 can compete with DNA polymerase α for binding to T antigen is the first report of a direct effect of p53 on the biochemical activity of T antigen. If the formation of a complex between T antigen and DNA polymerase α is an essential step in SV40 DNA synthesis then p53 could potentially block this process. The p53 from non-permissive species binds more tightly to T compared to p53

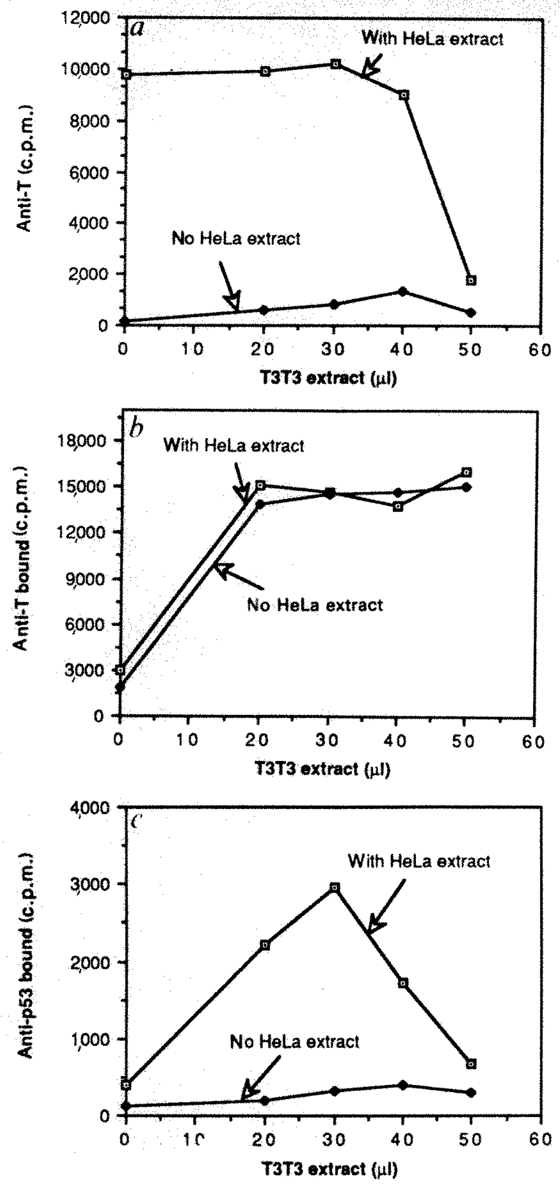

Fig. 4 Simultaneous analysis of the formation of T-pol, T-p53 and a trimeric pol-T-p53 complex. Extracts of T3T3 cells were mixed at different concentrations with extracts of HeLa cells or buffer alone and incubated with pure T antigen. Aliquots of the mixtures were assayed for the presence of T-pol complexes, T-p53 complexes and pol-T-p53 complexes. *a*, T-pol complex assay using an anti-T label (PAb 419) and an anti-DNA polymerase α (SJK 287-32) as solid phase. *b*, T-p53 complex assay using the same anti-T label as in *a* and an anti-p53 (RA3 2C2, ref. 21) as solid phase. *c*, pol-T-p53 complex assay using an anti-p53 (200.47, ref. 22) as label and an anti-DNA polymerase α (SJK 287-32) as solid phase.

Methods. Assay conditions and wash steps are described in Figs 1, 2 and 3. A range of volumes of T3T3 extract (0 μl, 120 μl, 180 μl, 240 μl and 300 μl) prepared in HYPO buffer were adjusted to a final volume of 300 μl in the same buffer. An equal volume of HeLa extract or buffer (no HeLa extract) was added and pure T antigen added to each mixture. After incubation two 100 μl aliquots of each reaction mix were assayed in each of the three assays.

from permissive species[16], and competition between p53 and DNA polymerase α for T could be a determinant of host permissiveness.

Indeed we recently learned that transfection of murine (but not human) p53 expression plasmids into COS cells leads to a dramatic reduction in the T-dependent replication of cotransfected SV40 origin containing plasmids. These results (J. Jenkins, personal communication) provide a striking *in vivo* correlation to the *in vitro* studies presented here.

The anti-pol antibodies used in this study will, under certain assay conditions, react with murine DNA polymerase α. T will bind murine DNA polymerase α *in vitro*, provided the extracts have been depleted of murine p53, and as this association is also blocked by p53 (D.P.L. and J.V.G., manuscript in preparation), the T-pol interaction may be important in viral transformation as well as in lytic infection. The connection between p53 and DNA polymerase α resulting from their competitive binding to SV40 T antigen could provide insight into the normal cellular function of the p53 proto-oncogene. Possibly a cellular inhibitory factor that binds to DNA polymerase α might also bind p53. The increased amounts of p53 found as cells enter S phase[17] could be necessary to remove this factor, and the high p53 concentrations found in a range of tumour cells might be causally linked to their uncontrolled DNA replication.

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Mouse p53 inhibits SV40 origin-dependent DNA replication

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p53 is a cellular phosphoprotein that is present at elevated concentrations in cells transformed by different agents[1-5]. p53 complementary DNA expression-constructs immortalize primary cells *in vitro*[6,7] and co-operate with an activated *ras* oncogene in malignant transformation[6,8,9]. Several reports have implicated p53 in mammalian cell cycle control and specifically with events occurring at the G_0–G_1 boundary[10,11]. p53 forms specific complexes with simian virus 40 (SV40) large-T antigen[1,2], and such complexes are found associated with both replicating and mature SV40 DNA in lytically infected cells[12]. In an accompanying paper Gannon and Lane[13] report that in *in vitro* plate-binding assays, mouse p53 can displace polymerase α from complex with T-antigen. We have examined the *in vivo* consequences of expressing wild-type and mutant p53 proteins from other species in SV40-transformed

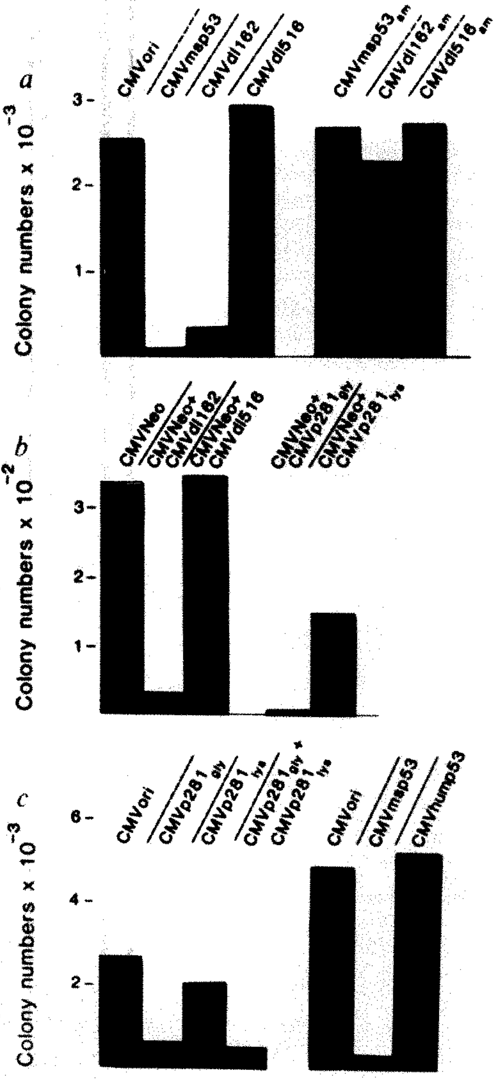

Fig. 1 Mouse p53 inhibits SV40 origin-dependent DNA replication.

Methods. Monkey COS cells are seeded from confluent flasks into 60 mm tissue culture dishes in Dulbecco's modified Eagles's medium (DMEM; Flow Laboratories) supplemented with 10% fetal calf serum (FCS; Flow Laboratories) and L-glutamine. 2 h later cells are washed in DMEM minus FCS and transfected with 5 µg of plasmid DNA per dish using the DEAE/Dextran procedure[24]. After a further 4 h, cells are treated with Hank's buffered saline containing 10% dimethylsulphoxide for 2 min. Cells are washed and then incubated for 72 h in DMEM + 10% FCS at 37 °C with 10% CO_2, washed with phosphate-buffered saline (PBS) and the plasmid DNA isolated using modified Hirt supernatant procedures[30]. Plasmid DNA is digested with excess *Dpn*I restriction endonuclease (New England Biolabs). Parent and replicated daughter DNA molecules are distinguishable by their differential sensitivity to the methylation sensitive enzyme *Dpn*I (ref. 16). The restriction enzyme digestions are used to transform competent DH5 *E. coli*[31]. *Dpn*I resistant DNA molecules representing the replicated fraction transform *E. coli* efficiently and are thus a measure of plasmid replication[16]. Transformation efficiency was about 10^7–10^8 bacterial colonies per µg input plasmid. Cells are plated on L-agar supplemented with 0.01 M $MgCl_2$ and 50 µg ml^{-1} ampicillin or kanamycin, and incubated overnight at 37 °C. Colonies are counted manually. Plasmids: all p53 cDNA expression vectors used in the present study were constructed in the pBC12CMV vector described by Cullen[14]. This contains the 200 base pair *Sph*I to *Hind*III restriction fragment from SV40 DNA which encompasses a functional viral DNA replication origin. CMVori lacks an expressed p53 cDNA gene; CMVmsp53 encodes wild-type mouse p53; CMVdl162 and CMVdl516 contain internal deletions of mouse p53 (see ref. 7); CMVmsp53$_{am}$, CMVdl162$_{am}$ and CMVdl516$_{am}$ each contain an additional amber mutation at residue 609 (ref. 20) which causes translation to terminate at the amber codon, giving rise to a truncated N-terminal peptide (J.R.J. and C.P., manuscript in preparation); CMVNeo encodes the Tn5 gene product that confers resistance to the antibiotic G418 in animal cells, and to the neomycin-kanamycin family in *E. coli*[21]; CMVp281$_{gly}$ and CMVp281$_{lys}$ express mouse p53 proteins in which the normal amino acid at position 281 has been converted respectively from glutamic acid to glycine and lysine (C.A. and J.R.J., in preparation). In several experiments, parallel transfections were carried out in which cells were fixed and examined for p53 expression by indirect immunofluorescence. Using separately the murine specific monoclonal antibodies pAB242 and pAB248 (ref. 18) and the rodent specific monoclonal antibody pAB200-47 (ref. 19), CMVdl516, CMVmsp53 and CMVdl162 all showed ~30–40% p53 positive cells.

monkey cells. We report here that expression of mouse p53 results in a substantial and selective inhibition of SV40 origin-dependent DNA replication. In addition to any function in the G_0–G_1 transition, the data presented suggest that p53 may affect directly the initiation or maintenance of replicative DNA synthesis.

Replication experiments were carried out by transfecting SV40 origin-containing plasmids into the SV40-transformed monkey COS cell line[15] which is permissive for SV40 replication. The plasmids are described in the legend to Fig. 1. Figure 1*a* shows that plasmids CMVmsp53 and CMVdl162 are restricted in their ability to replicate whereas CMVdl516 compares with the control CMVori. In five independent experiments, CMVmsp53 replicated on average only 4% compared with CMVori, and CMVdl162 remained slightly (3 to 4 times) higher than CMVmsp53. In another set of experiments, COS cells were transfected with wild-type and mutant p53 expression constructs containing an additional amber mutation at residue 609. The results of these transfections are also shown in Fig. 1*a*. The amber mutations give complete restoration of the replicative capacity of both CMVmsp53 and CMVdl162. CMVdl516$_{am}$ remains essentially identical to CMVdl516. We conclude that expression of a full-length encoded protein product is required for restriction of DNA replication. p53 expression constructs were next co-transfected into COS cells together with the construct CMVNeo, which encodes kanamycin resistance. The results (Fig. 1*b*) show that CMVdl162 suppresses replication of the cotransfected CMVNeo, whereas CMVdl516 has no effect. Similar results were obtained with two point mutants of p53 (see below, and Fig. 1*b*). We conclude that the restriction of replicative capacity shown by CMVdl162 operates in *trans* and, together with the results of the amber mutant experiments, argues that it is directly due to the encoded mouse p53 protein.

To eliminate the involvement of some non-specific toxic effect, COS cells were transfected with CMVori, CMVmsp53 or CMVdl516 and these cell populations examined by flow cytometry[22]. The results (Fig. 2) show that there is no detectable alteration in the distribution of DNA contents. Similar results were obtained by pulse-labelling replicating DNA with ^{3}H-thymidine (data not shown). We conclude that there is no significant inhibition of cell cycle progression or of cellular DNA synthesis by the mouse wild-type cDNA gene product, and that restriction of replicative synthesis is therefore SV40 origin-specific. Western blotting shows that there is no relationship between steady state levels of the expressed p53 proteins and inhibition (data not shown).

The mouse p53 expresssion-constructs described here fall into two classes: those encoding a T antigen-binding protein inhibit plasmid replication, but those encoding a non-T antigen-binding

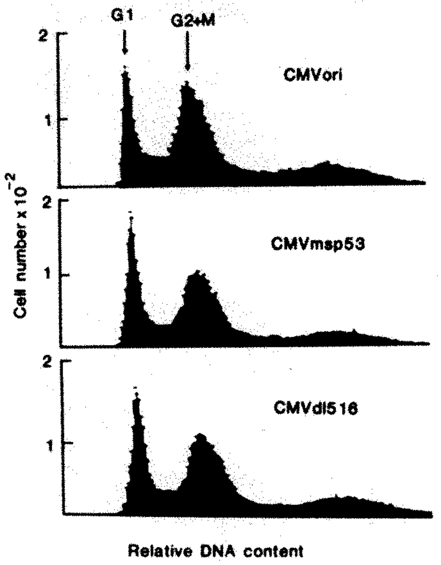

Fig. 2 Mouse wild-type and mutant p53s do not inhibit proliferation of transfected monkey COS cells. Cells were transfected as described for Fig. 1. 72 h post-transfection, cells were trypsinized and fixed in cold (−20 °C) 70% ethanol and stored at −20 °C. Just before analysis of DNA content by flow cytometry, fixed cells were briefly pelleted then resuspended in PBS and incubated with 100 μg ml^{-1} ribonuclease A for 30 min at 37 °C, and the DNA stained with propidium iodide[22]. Under the conditions of the experiments described here in which 30-40% of cells were p53 positive by immunofluorescence (see legend to Fig. 1), previous work with a similar proportion of antigen positive cells showed substantial alterations in cell cycle progression by flow cytometry[23]. The cell cycle phases G1, G2 and M (mitosis) are indicated.

protein[24,25] do not. Two point mutants of mouse p53 were tested for their effects on plasmid replication: CMVp281$_{\text{gly}}$ p53 which binds T-antigen, and CMVp281$_{\text{lys}}$ p53, which does not. The results are shown in Fig. 1*c*. CMVp281$_{\text{gly}}$ inhibits plasmid replication but CMVp281$_{\text{lys}}$ does not, and in cotransfections inhibition is dominant (Fig. 1*c*). To determine if inhibition of replication is a general feature of T antigen binding-p53 protein overexpression, we made and transfected an expression construct encoding a full-length human p53 protein. Human p53 also binds T-antigen in these assays. Fig. 1*c* shows the result. Although the human protein is efficiently expressed, is detectable by indirect immunofluorescence in an equivalent percentage of the transfected cell population, and accumulates at high levels in such cells, there is no detectable inhibition whatsoever of SV40 origin-dependent replicative synthesis. It appears therefore that suppression of such replicative activity is a characteristic of the murine p53 protein and is not a general feature of the T/p53 interaction *per se*.

SV40 DNA replication requires the virally encoded T antigen[26] as well as a range of host cell proteins[29] and T-antigen/polymerase α complexes appear to be required for SV40 replication *in vitro*[27]. p53 also forms complexes with large T antigen[1,2] and in an accompanying paper[13] it is demonstrated that mouse p53 can displace polymerase α from T-antigen in *in vitro* plate-binding assays. Taken in isolation, the mouse p53 data we present here, along with the observations of Gannon and Lane[13], suggest that p53 might function as a regulatory factor controlling, for example, the recruitment of SV40 chromosomes for cycles of replicative synthesis. The human p53 result (Fig. 1*c*) suggests otherwise, however. It seems more likely that inhibition of replication by mouse p53, and perhaps DNA polymerase α displacement, also results from some intrinsic species difference between the way mouse and human (and presumably monkey) p53 interact with T antigen. For example, in SV40 permissive cells it could be DNA polymerase α that displaces p53 from a complex with T-antigen, and that p53 participates in some intermediate step between T-antigen binding and DNA polymerase α-mediated synthesis. It is significant that mouse cells are non-permissive for SV40 replication whereas human cells are semipermissive[28], and SV40 replicates poorly in HeLa cells that lack a normal p53 protein. HeLa cell extracts do support SV40 DNA replication *in vitro*[16,17], yet not as efficiently as other human cell extracts[17]. We suggest that differences between mouse and human p53 are able to play a part in defining viral host range. A number of reports have implicated p53 in early events following the stimulation of quiescent cells, and specifically in the transit from G_0 to G_1[10,11]. We suggest that p53 could also be directly involved in the initiation or maintenance of replicative DNA synthesis.

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The 5S gene internal control region is B-form both free in solution and in a complex with TFIIIA

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Rhodes and Klug[1] have recently proposed that the internal control region of the *Xenopus* 5S RNA gene adopts an A-type DNA structure in solution. This suggestion was based on a Fourier analysis of both the spacing of DNase I cutting sites and on the distribution of G residues in the DNA sequence. Both revealed a ~5.6–5.7-base periodicity which the authors interpreted as a structural repeat every half helical turn of A-type DNA. This contention was strengthened by the finding that a 9-base-pair (bp) double-stranded deoxyoligonucleotide corresponding to residues +81 to +89 of the 5S gene exhibits an A′ RNA-like crystal structure[2].

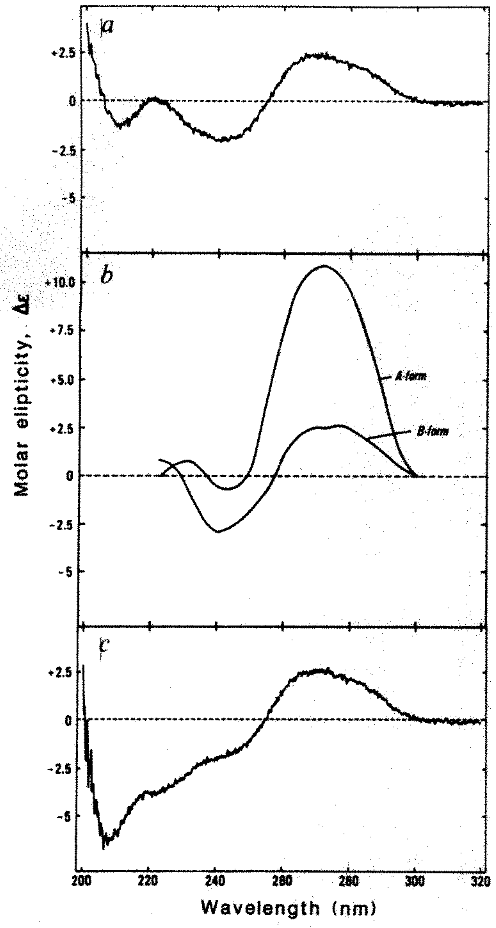

Fig. 1 Circular dichroism spectra of 5S DNA (*a*), B- and A-form reference spectra (*b*) and 5S DNA complexed with TFIIIA (*c*). CD spectra were obtained at a DNA concentration of 68 μg ml^{-1} (*a*) and 49 μg ml^{-1} (*c*) in 50 mM KCl, 10 mM Tris-Cl, *p*H 7.5, 5 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 0.1 mM dithiothreitol using a 0.2-cm optical path length. Spectra were recorded with an AVIV 61DS spectropolarimeter scanning from 320 to 200 nm with data collection every 0.25 nm. The spectra shown (*a* and *c*) are the average of five independent measurements with base line corrected for the buffer alone. The molar elipticity is plotted to take account for differences in DNA concentration. The reference spectra were taken from Sprecher *et al.*[8].

Methods. Two 58-bp oligonucleotides were synthesized with an Applied Biosystems DNA synthesizer. These DNAs correspond in sequence to nucleotides +40 to +97 of the noncoding strand of the *X. laevis* 5S RNA gene[19] and nucleotides +44 to +97 of the coding strand plus an additional four nucleotides of sequence 5'GATC3'. The oligonucleotides were deblocked and purified by gel electrophoresis. Equimolar amounts, estimated from absorbance at 260 nm, were mixed in a solution containing 0.1 M $NaHCO_3$, *p*H 8.0, and heated to 95 °C for 5 min and allowed to reassociate by slow cooling to 22 °C over 3-4 h. The duplex DNA was precipitated with ethanol, vacuum dried and resuspended in TFIIIA binding buffer (above). More than 97% of the DNA was double stranded as estimated by gel electrophoresis of an aliquot. TFIIIA was purified from 7S RNP particles from immature oocytes[6], and separated from 5S RNA and other proteins by DEAE cellulose chromatography, ribonuclease A digestion and Bio Rex 70 chromatography[13]. The protein was homogeneous as judged by silver staining of SDS polyacrylamide gels. TFIIIA (120 μg) was added to 34 μg of the DNA in a final volume of 700 μl providing a 3.5 molar excess of TFIIIA over DNA. The sample was incubated at 22 °C for 40 min before recording the CD spectrum over the course of 45 min at 23 °C. SDS gel electrophoresis demonstrated that the TFIIIA remained intact during this incubation. Likewise, electrophoresis under nondenaturing conditions showed that the complex remained intact.

This region of DNA is of special interest as it forms the binding site for the 5S gene-specific transcription factor IIIA (TFIIIA)[3]. TFIIIA is a Zn^{2+}-binding protein which interacts with both the internal control region of the gene and the 5S transcript[4-6]. As base-paired regions of RNA are of the A type[2,7], it was reasonable to postulate that 5S DNA might also adopt this conformation[1]. We report here that the circular dichroism (CD) spectrum of a synthetic 54-bp deoxyoligonucleotide corresponding to the TFIIIA binding site is similar to the CD spectrum of B-form DNA in solution. Further, DNA–TFIIIA complexes show an unaltered DNA CD component indicating no gross alteration in DNA structure on protein binding.

CD spectroscopy in the ultraviolet provides a very sensitive method of examining alterations in DNA conformation[8]. The magnitude of the positive CD band at 275 nm is highly dependent on helix winding angle and on base-pair twist[9], two parameters which differ dramatically between the B and A types of DNA[10]. Correlations between X-ray structures of DNA and solution CD measurements are possible as CD spectra have been obtained from DNA films under the conditions of ionic strength and relative humidity used in the X-ray fibre diffraction studies of B- and A-form DNA[11]. Figure 1*a* shows the CD spectrum of a synthetic 54-bp double-stranded deoxyoligonucleotide which corresponds in sequence to residues +44 to +97 of the *Xenopus laevis* 5S RNA gene. For comparison the CD spectra of B-form and A-form DNAs are shown in Fig. 1*b* (ref. 8). The oligonucleotide spectrum largely resembles that of B-form DNA in low ionic strength solutions or in wet films[11]; however, there are some minor differences which are probably related to the fact that the 5S spectrum is of a unique sequence while the B-DNA spectrum is of calf thymus DNA and represents an average of all sequences in the genome. More importantly, major features are shared by both spectra: the magnitude of the major positive peak in both spectra is 2.3-2.5 (°l mol^{-1} cm^{-1}). The major positive and negative peaks are similar in magnitude in both the 5S and B-form DNA spectra (Fig. 1*b* and refs 8 and 11). This is clearly not the case for A-form DNA where the positive peak is approximately ten times more intense than the negative[8].

To demonstrate further that the 5S DNA spectrum obtained in aqueous solution reflects a B-type DNA structure, we examined the effect of solvent composition on the spectrum. Figure 2 shows that the 5S DNA oligonucleotide exhibits an A-type spectrum in 80% ethanol; further, this spectral transition occurs between 70 and 80% ethanol in both 5S DNA (not shown) and in bulk genomic DNA[8,11]. We previously measured the helical repeat of 5S DNA in solution using the topoisomer bandshift method[12] and found a value of ~10.6 bp per helical turn over the region of +28 to +97 of the 5S gene[13]. This value is typical of DNA in the B family of structures[12,14]. Taken

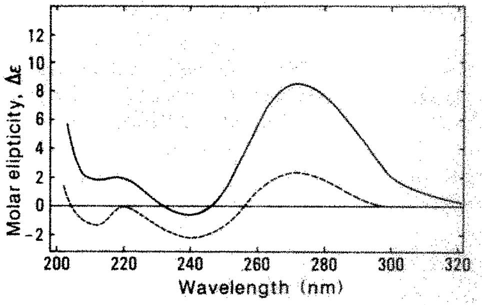

Fig. 2 B → A type transition in 5S DNA. CD spectra of the 54-bp double-stranded oligonucleotide were obtained in aqueous solution (10 mM Tris-Cl, *p*H 7.5, 0.1 mM EDTA, dashed line) and in 80% (v/v) ethanol (solid line). The DNA in aqueous solution was dialysed against steps of 25%, 50% and 80% (v/v) ethanol at 22 °C and insoluble material was removed by centrifugation. Molar elipticity was calculated based on the absorbance of the supernatant at 260 nm.

together, these data and the present CD measurements support the view that the 5S internal control region is B-type in low ionic strength aqueous solution.

We have also determined the CD spectrum of the 54-bp oligonucleotide in a complex with TFIIIA (Fig. 1*c*). In this experiment, a ratio of 3.5 molecules of TFIIIA per 5S DNA molecule was used to ensure that all DNA molecules were complexed with protein. The spectrum of the complex above 240 nm is largely unchanged from that of the protein-free DNA. Below 240 nm the protein greatly influences the spectrum. These data suggest that no major structural change such as B→A transition occurs in the DNA upon TFIIIA binding.

These data are thus in agreement with the results of nuclease digestions on the TFIIIA-DNA complex where it has been found that the periodicity of DNase I cleavage within the TFIIIA binding site is ~10.0-10.4 bases[13,15]. If this spacing reflects the helical repeat of the DNA in the complex, then this supports the view that TFIIIA binds DNA in a B-type structure. If no change in helical repeat occurs on TFIIIA binding then previous measurements of change in linking number of TFIIIA binding[16] must indicate bending or wrapping of the DNA about the protein. Thus, our present results indicate that TFIIIA can recognize and bind to both B-type DNA and to A-type RNA[17].

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Spatial relationship of the Fis binding sites for Hin recombinational enhancer activity

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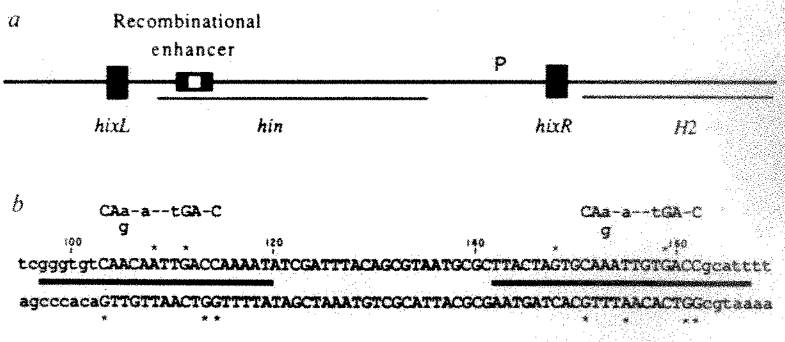
Site-specific recombination reactions involve the joining or rearrangement of discrete DNA segments in a highly precise manner. A site-specific DNA inversion regulates the expression of flagellin genes in *Salmonella* by switching the orientation of a promoter[1,2]. Analysis of the reaction has shown that, in addition to DNA sequences at the two boundaries of the 1-kilobase invertible segment where strand exchange occurs, another *cis* acting sequence is required for efficient inversion[3]. This 60-base-pair enhancer-like sequence can function at many different locations and in either orientation in a plasmid substrate. It includes two binding sites for a host protein called Factor II or Fis (refs 4 and 5). Here we have investigated the importance of the spatial relationship between the two Fis binding sites for enhancer activity and have found that the correct helical positioning of the binding sites on the DNA is critical. However, this result could not be accounted for by effects on Fis binding. We propose a model for enhancer function in which the enhancer region acts to align the recombination sites into a specific conformation required for productive synapsis.

The inversion reaction can be performed in a purified *in vitro* system derived from *Escherichia coli*[4]. The Fis protein and two other proteins, Hin and HU, are required for maximum rates of inversion. The Hin protein, which is encoded by sequences within the invertible segment (see Fig. 1*a*), interacts specifically with the DNA sequences at the two 26-base-pair (bp) recombination sites (*hixL* and *hixR*). Hin is a member of a family of prokaryotic site-specific recombinases which promote DNA inversion, including the Gin (refs 6 and 7) and Cin (ref. 8) systems which control tail fibre expression in bacteriophage Mu and P1, respectively, and the Pin system[9] whose function is unknown. The HU protein interacts nonspecifically with DNA and shows the greatest stimulatory effect on the reaction when the enhancer is located within 350 bp of the closest recombination site[4].

The recombinational enhancer sequence is normally located 103 bp from *hixL* (Fig. 1*a*), although it can function on either side of the recombination sites and as far as 4 kilobases (kb) from the closest recombination site[3]. Recombination rates of plasmid substrates lacking the enhancer are <0.5% of those found for plasmids containing enhancers. The nucleotide sequences responsible for enhancer function have been previously mapped by deletion and substitution analysis to extend from nucleotide +103 to +163 (as measured from the centre of *hixL*, see Fig. 1). Within this 60-bp sequence, the enhancer is divided into two domains referred to as the proximal and distal domains with respect to *hixL*. Footprinting and chemical interference experiments have demonstrated that the Fis protein binds to these two domains (Fig. 1*b*)[24]. Fis binds with ~fivefold higher affinity to the *hixL* distal domain than to the *hixL* proximal domain.

To investigate the importance of the relative position of the two Fis binding sites for enhancer function, we constructed mutant enhancers containing increasing numbers of base pairs between the two domains. The insertions are all located at the *Cla*I restriction site between the proximal and distal domains and range in size from 2 to 43 bp (Fig. 1*c*). The mutant substrate DNAs were assayed in the reconstituted *in vitro* system[4]. Figure 2 shows the data for reaction times of 3 and 30 min. Under the conditions used in these experiments, the wild-type substrate pMS551 generates 50% of the final yield of recombinant molecules after ~2 min and the maximum yield of recombinants after ~15 min (data not shown). The longer incubation times were used for the data presented in Fig. 2 to enable the lower reaction rates found in the mutants to be measured.

Increasing the spacing between the enhancer domains by two base pairs (pMS553) results in an 80% decrease in the number of recombinants measured after a 3-min incubation period as compared with the wild-type substrate. After 30 min, slightly over half the wild-type number of recombinants have accumulated with pMS553. An insertion of 5 bp, or half a helical turn of DNA, abolishes enhancer activity as pMS680 recombines at a rate that is slightly lower than a plasmid in which the proximal domain of the enhancer is deleted (pMS609). However, an insertion of 10 bp (pMS552), or approximately one complete turn of the DNA helix, largely restores enhancer function (about 70% of the wild-type rate after 3 min). Plasmid pRJ719, which also contains a 10-bp insertion but with a different sequence from pMS552 (Fig. 1*c*), recombines at about 45% of the wild-


a
Recombinational
enhancer
P
hixL
hin
hixR
H2
b
CAa-a--tGA-C
g
100
120
140
150
tcgggtgtCAACAATTGACCAAAATATCGATTTACAGCGTAATGCGCTTACTAGTGCAAATTGTGACCgcatttt
agcccacaGTTGTTAACTGGTTTTATAGCTAAATGTCGCATTACGCGAATGATCACGTTTAACACTGGcgtaaaa

c

Plasmid	Number of base pairs inserted	Sequence of insert
pMS551	0	TATC[]GATT
pMS553	2	TATC[GC]GATT
pMS680	5	TATC[GAGGC]GATT
pMS552	10	TATC[GCCTCGAGGC]GATT
pRJ719	10	TATC[GTTCTAGAAC]GATT
pMS673	14	TATC[GCCGGAATTCCGGC]GATT
pMS684	18	TATC[GCCGGAATTAATTCCGGC]GATT
pMS696	21	TATC[GCCTCGATCGACGATCGAGGC]GATT
pMS660	24	TATC[GATGCGATGTTTCGCTTGGTGGTC]GATT
pMS681	24	TATC[GCCGGAATTCCATGGAATTCCGGC]GATT
pMS683	27	TATC[GCCGGAATTCCATCATGGAATTCCGGC]GATT
pMS662	30	TATC[GACTTCGGCGACCGCGGCTGGCTGGCCATC]GATT
pMS695	32	TATC[GCCTCGATCGACGATCGATCGTCGATCGAGGC]GATT
pMS698	43	TATC[GCCTCGATCGACGATCGATCGACGATCGATCGACGATCGAGGC]GATT

Fig. 1 *a*, Structure of the invertible DNA segment controlling *H2* expression in *Salmonella*. The *hin* gene, the left and right recombination sites (*hixL* and *hixR*), and the recombinational enhancer are shown. P, approximate position of the promoter which transcribes *H2* when oriented in the ON orientation as shown here. *b*, Nucleotide sequence around the Hin recombinational enhancer[3]. Bar, boundaries of the sequences from both strands that are protected from DNase I cleavage by Fis in footprinting experiments; *, purine which when methylated by dimethyl sulphate prevents binding by Fis[24]. Shown above the sequence is a possible recognition sequence for Fis binding. *c*, Plasmids containing mutant enhancers: pMS551 contains the wild-type enhancer region[3]; the other plasmids are derivatives of pMS551 and contain the indicated number of base pairs inserted in the *Cla*I site at position 122. The sequence of the inserted DNA is shown in brackets.

Methods. Plasmid pMS551 has *hixL* and the enhancer region (positions −99 to +207) between the *Eco*RI and *Hin*dIII sites and a synthetically-derived 26-bp *hixL* site cloned into the *Sal*I site of pBR322 (ref. 3). The construction of pMS553 and pMS552 has also been described[3]. Plasmid pMS680 was made by digesting pMS552 with *Xho*I, which cuts within the inserted DNA, and digesting with nuclease S_1 (10 min at 30 °C with 1 unit S_1 nuclease (Pharmacia) per μg DNA) followed by blunt end ligation; pRJ719 was made by digesting pMS551 with *Cla*I and ligating in the presence of a 500-fold molar excess of the oligonucleotide pCGTTCAGAA. Plasmid pMS673 was constructed as follows: pMS551 was cleaved with *Eco*RI, the protruding ends filled in with T4 DNA polymerase and dNTPs, and religated to destroy the *Eco*RI site. This plasmid (pMS672) was then digested with *Cla*I, filled in with T4 DNA polymerase and dNTPs, and the *Eco*RI-site containing linker CCGGAATTCCGG was inserted to give pMS673. Plasmid pMS684 was derived from pMS673 by digestion with *Eco*RI, filling in with T4 DNA polymerase and dNTPs, and blunt end ligation; pMS696, pMS695 and pMS698 were constructed by ligating *Xho*I-cleaved pMS552 in the presence of a 500-fold molar excess of the oligonucleotide pTCGATCGA/TCGA giving rise to plasmids containing between one and three inserts of the oligonucleotide, respectively; pMS660 and pMS662 were made by ligating a 24-bp and 30-bp, respectively, *Taq*I fragment from pRZ102 (colE1::Tn5, ref. 19) into *Cla*I cleaved pMS551; pMS681 was constructed by ligating the *Nco*I-site-containing oligonucleotide AATTCCATGG into *Eco*RI digested pMS673; and pMS683 was made by digesting pMS681 with *Nco*I and filling in the protruding ends with T4 DNA polymerase and dNTPs followed by blunt end ligation. Sequence analysis indicated that only three of the four bases of each end were filled in, with one base deleted, giving rise to the sequence shown for pMS683. The nucleotide sequence of the entire enhancer region for each of these plasmids was confirmed by direct plasmid dideoxy sequencing[20] using an oligonucleotide primer from +71 to +90.

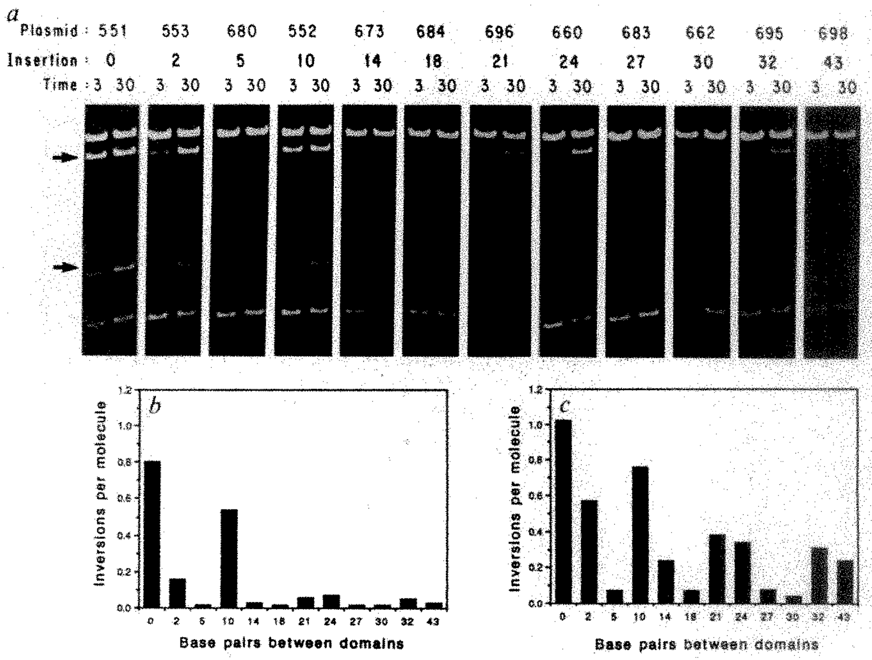


Fig. 2 *a*, Inversion assays *in vitro* of enhancer spacer mutants. Plasmid DNA was incubated at 37 °C with purified Hin, Fis and HU for 3 and 30 min as described[4], then the mixture was digested with *Pst*I and *Hin*dIII and analysed by agarose gel electrophoresis. Arrows, inversion products. The substrate plasmid, number of base pairs inserted, and incubation times are given above the gels. *b*, *c*, Bar graphs depicting the number of inversions per DNA substrate molecule for each plasmid. *b*, 3-min and *c*, 30-min assays. The proportion of molecules in the inverted orientation was determined by scanning photographic negatives with a laser densitometer and the number of inversions per DNA molecule was calculated[21]. Not shown are the results of pMS681 (+24), which gave essentially identical values as pMS660 (+24), and pMS609, which is deleted for the proximal domain of the enhancer[3] and which gave <0.02 and 0.08 inversions per molecule after 3 and 30 min, respectively.

type rate and accumulates about 90% of the wild-type number of recombinants after 30 min. Insertions of 14 and 18 bp progressively decrease enhancer function whereas a 21-bp insertion, or two complete turns of the helix, results in significantly higher rates. This helical periodicity for enhancer function is maintained, albeit with very low reaction rates, for three (32 bp) and probably four (43 bp) turns of the DNA helix. The recombination assays clearly indicate that the helical orientation of the two Fis binding sites in the enhancer is critical for function. Moreover, an increase of spacing of one complete turn has only a small effect on enhancer function, but two or more turns are poorly tolerated.

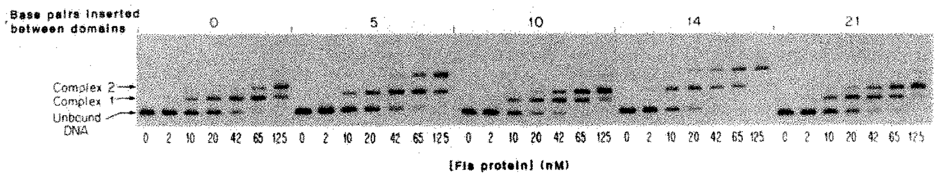


Fig. 3 Polyacrylamide gel electrophoresis of Fis/enhancer DNA complexes. A 276-bp *Hin*dIII-*Eco*RV restriction fragment from pMS551 containing the wild-type enhancer sequence and the corresponding *Hin*dIII-*Eco*RV restriction fragments from pMS680 (+5), pMS552 (+10), pMS673 (+14) and pMS696 (+21) were isolated after end-labelling at the *Hin*dIII site using the Klenow fragment of DNA polymerase and [α-^{32}P]dATP. Binding and electrophoresis of the Fis/enhancer DNA complexes were as described[24]. The binding reactions were performed in 80 mM NaCl, 25 mM Tris-HCl, *p*H 7.9, 1.2 mM EDTA, 2 mM dithiothreitol, 0.5 mg ml^{-1} bovine serum albumin, 0.2 mg ml^{-1} polycytidylic acid and 15 μg ml^{-1} sonicated calf thymus DNA. Electrophoresis times and conditions were identical for each titration.

Studies of repressor and activator interactions in several systems have shown the importance of correct helical orientation of protein binding sites[10-12]. For example, λ repressor binding to variably spaced operator sites demonstrated that correct positioning on the helix was necessary to allow for cooperative interactions to occur between repressor proteins[11]. In an analogous fashion, the helical dependence seen for Fis activity in recombination could reflect cooperative interactions between Fis proteins bound at the two domains of the enhancer. However, measurements of Fis binding show no evidence of cooperative interactions. Binding of Fis to the enhancer domains of the wild-type and selected mutant enhancers was measured using gel retardation assays[22,23] (Fig. 3). Titration of Fis protein with a constant concentration of wild-type enhancer-containing restriction fragment revealed two bound species (complex 1 and complex 2 in Fig. 3). Complex 1 appears at low protein concentrations and consists of Fis bound predominantly at the distal domain; complex 2 contains Fis bound to both the proximal and distal domains[24]. The insertion mutants show little differences in the formation of these complexes as a function of protein concentration. Additional experiments, where protection from DNase I cleavage was measured as a function of added Fis protein, resulted in no differences in affinity or footprinting patterns that correlated with the helical disposition of the Fis binding domains (data not shown). Although all these experiments are on linear DNA, the results suggest that the requirement for correct helical positioning of the enhancer domains on the DNA for Fis activity is not the result of cooperative interactions between Fis proteins which facilitate binding. This conclusion is further supported by the finding that DNA fragments containing isolated proximal or distal enhancer domains bind Fis with the same relative affinity as the wild-type fragment containing both domains[24]. However, we cannot rule out the possibility that the helical dependence on recombination is a function of cooperative binding effects that only occur on superhelical DNA.

We have observed that the relative mobilities of the complexes in the gel retardation assay are different depending on the helical position of the Fis binding sites. Complexes formed with pMS680 (+5) and pMS673 (+14) enhancer DNA fragments show a greater electrophoretic shift than do complexes formed with pMS551 (wild-type), pMS552 (+10), and pMS696 (+21) DNA fragments (Fig. 3). The different mobilities may reflect unique structural features of the enhancer DNA[13]. DNA fragments containing mutant enhancers without bound Fis also migrate aberrantly on polyacrylamide gels (data not shown), suggesting deformations associated with the DNA helix. Binding by Fis protein may generate further structural changes in the DNA. Structural perturbations such as bends or kinks in the DNA would lead to altered relative mobilities depending on whether they are oriented towards, or away from, each other[14]. Thus, similar electrophoretic shifts are obtained with complexes generated with enhancer fragments that contain integral numbers of DNA helical turns between proximal and distal domains, whereas different shifts are obtained with fragments containing insertions of a non-integral number of helical turns (Fig. 3).

Figure 4 shows a model for the function of the enhancer in site-specific DNA inversion. Hin and Fis bind independently to their respective recognition sites on the DNA. Protein-mediated interactions between Hin and Fis then occur which result in looping out of the intervening DNA between each recombination site and the enhancer. In this manner, the Hin molecules and recombination sites are assembled into the proper synaptic complex to give inversion of DNA after strand exchange.

A number of features of this model are consistent with the properties of the Hin enhancer. The enhancer/recombination site complex could assemble without regard to the precise location or orientation of the enhancer relative to the recombination sites. The only consideration in this respect is that there be sufficient distance between the protein binding sites for the DNA to be able to loop around to achieve the required protein interactions. Consistent with this, we have previously shown that enhancer cannot function when it is very close (within 30 bp) to one of the recombination sites[3]. We have also found that the major enhancing effect of the HU protein on the reaction occurs when the enhancer is within ~200 bp of the nearest recombination site[4]. The HU protein may be promoting or stabilizing the required bending of the DNA, and thus its stimulatory effect is greatest when the enhancer and recombination sites are close to each other.

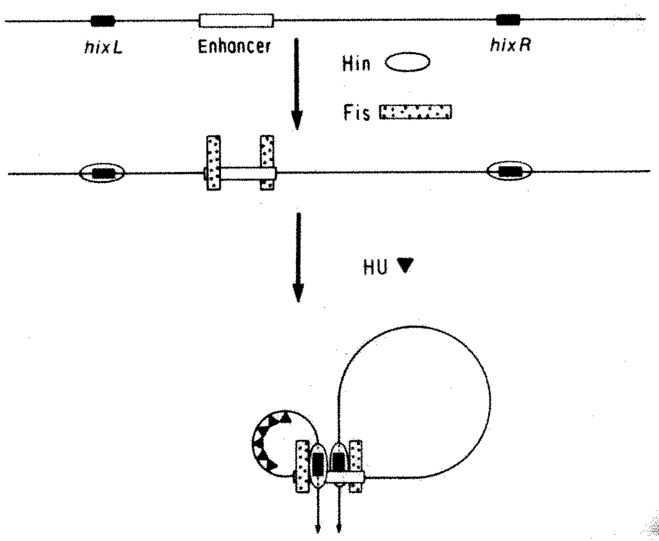


Fig. 4 Model for enhancer function in the formation of the synaptic complex. Subsequent to binding of Hin and Fis to their recognition sequences on the DNA, a tripartide nucleoprotein complex is assembled at the enhancer. The assembly of this complex could occur by independent association of each recombination site with an enhancer domain or, alternatively, Hin proteins could first pair the two recombination sites, followed by interaction with the enhancer.

In this 'looping-synapsis' model, the critical function of the enhancer is to position the recombination sites in exactly the correct configuration to complete synapsis. That enhancer function is dependent on the helical position of the Fis binding sites provides strong support for this idea. Separation of the enhancer domains by an additional half-turn abolishes enhancer activity, whereas function is regained when one full turn of the helix is inserted. Interactions which facilitate binding of Fis proteins at the two domains are probably not responsible for the observed helical dependence for enhancer activity because binding affinities measured on DNA fragments containing the altered spacing mutants are similar. Recent data suggest that bound Fis proteins are located in direct repeat orientation 48 bp from each other or ~4.6 turns of the helix[24]. Thus, in Fig. 4 we have shown Fis proteins binding to opposite sides of the helix. Further studies, including analysis of the structural properties of the enhancer DNA, will be required before the precise spatial relationship of the two Fis binding sites is known.

The looping-synapsis model accommodates many of the features that have been found for the Hin enhancer and provides a useful guide in thinking about enhancers in site-specific recombination. An important prediction of the model is that specific protein–protein interactions occur between the Hin recombinase and Fis. We are currently probing for such interactions. It is also worth noting that the related site-specific recombinase, resolvase, requires two additional binding sites which are adjacent to the site of recombination[15]. The Fis binding sites in the Hin inversion system may have a function similar to those of the additional resolvase binding sites in aligning the protein and DNA sequences at the site of recombination[16,17]. It seems likely that the assembly of complex nucleoprotein structures involving multiple protein binding sites on the DNA will be increasingly recognized as a feature of protein/nucleic acid reactions in which distantly spaced regulator or enhancer sequences are involved[18].

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Can the product of the θ gene be a real globin?

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A new member ($\theta 1$, or $\psi\alpha$) of the α-globin gene family has recently been identified in a number of species[1–5]. In higher primates the $\theta 1$ gene has all the structural features apparently necessary for expression, and it appears to have long been under strong selective constraints which suggests that it could still be, or recently have been, a functional gene[2]. No corresponding 'globin' has yet been identified, however. In some other species, galago[5] and rabbit[4] for example, the $\theta 1$ and $\psi\alpha$ genes have accumulated enough inactivating mutations for them to be considered genuine pseudogenes. Horses also have an α-like gene ($\psi\alpha$) (ref. 3), in a 3′ position identical to the other species in relation to the functional α genes, and this also appears to have the elements required for a functional gene. The predicted amino-acid sequence, however, suggests that any 'globin' product is likely to be non-viable because it has a number of seriously deleterious amino-acid replacements. Some of these amino-acid changes are shared with the rabbit and primate sequences, indicating that they predate the mammalian radiation, and that if indeed any of these genes are still functional, they are unlikely to be making haemoglobin.

The sequence of the equine $\psi\alpha$ gene (Fig. 1) has normal initiation and termination signals, correct splicing sequences, the same poly(A)-addition signal (AGTAAA) as the rabbit and primate genes[2,4], and lacks any obvious inactivating mutations. As in the primate genes, the promoter region is displaced ~150 base pairs to 5′ (bp) relative to its position in known functional α genes, and it lacks a canonical CCAAT sequence, the closest candidate being the ACAAT ~ 30 bp 5′ to the ATA sequence at nucleotide 64.

Comparison (Fig. 1) of this sequence with that of the adjacent $\alpha 1$ gene shows that 71 (50%) of codons have had base substitutions, of which 53 (37%) are changes (replacements) that would produce an amino-acid substitution. Moreover, there are 22 codons in the horse $\alpha 1$ sequence that can change to terminators by a single base change; 11 have sustained changes in $\psi\alpha$, but none to a terminator codon, whereas 3-4 might have been expected on a random basis in the absence of any selective constraints. When expressed as a corrected[6] percentage of the nucleotide sites that have had replacement (23%) or silent (44%) site changes, these figures are very similar to those seen in the orang-utan $\alpha 1$–$\theta 1$ comparison[2] (Table 1). In contrast, since the divergence of horses and orang-utans, their α genes have sustained only 9% replacement site changes compared with 40% silent site changes.

In globin genes and pseudogenes of various species, replacement sites appear to undergo substitutions at the rate of approximately 0.1×10^{-9} per site per year, and silent and non-coding sites at 5-7 times this rate[6,7]. Thus the 9% replacement site rate for the horse/orang α gene comparison equates roughly to their divergence at around the time of the mammalian radiation ~85 Myr ago. Applying similar considerations to the horse and

Table 1 Per cent corrected divergence of gene sequences

	Replacement sites	Silent sites
α genes		
Horse versus orang-utan	9	40
Rabbit versus human	12	29
Horse versus human	7	40
$\psi\alpha$ (θ) genes		
Horse versus orang-utan	12	23
Rabbit versus orang-utan	14	26
α versus $\psi\alpha$ (θ)		
Horse	23	44
Orang-utan	26	45

See ref. 6.

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CCAGAATGCGTGCTAAAATTCATGTCATCTACTAACAATAATACACTTTAAATATTCGTT
TCCATACAGCAAACACCGCAAAAATGGCGGCCTCGGCGTCCAGGGTGATTGGAAGTGTGC   120
AGGCCGGAGCAGACGCTCAGTCGAGCGCTCCCGGACCCCTCGGGACTGGACTCTGCGAGC
AGAGGCGCGGCGCCCGCGGAGTGGCGCCCGCGGAGGCCGCGGACCCGCCGCCCAGGGGCG   240

                              AlaLeuAlaAlaAlaAspArgAlaThrVal
CAGGCGCGCTCGCGGGCCGGGCTCACGATGGCGCTGGCGGCGGCGGACAGGGCGACCGTG
                               T    T T  C  C    A A C A   C
                              Val   Ser         LysThrAsn

ArgAlaLeuTrpLysLysMetGlySerAsnValGlyValTyrAlaThrGluAlaLeuGlu
CGCGCGCTGTGGAAGAAGATGGGTAGCAACGTCGGCGTCTACGCGACCGAGGCCCTGGAG   360
AAG  CGCC    GT   G T  CG  C   CT    AG  T GCG A        A
Lys   Ala   Ser   Val   GlyHisAla   Glu   GlyAla

Ar
AGGTGCGGAGGCCCCGCGCCCTCCGGCTCGCCCTCCTTTGCTCCCCAAGCCCCTCCCGCC

                               gMetPheLeuGlyPheProSerThrThrT
CGGACGCACCTCTCTCGCGTTCCTCCTGCAGGATGTTCCTGGGCTTCCCCTCCACCACGA   480
                                                  A      A
                                                  Thr   Lys

hrTyrPheLeuHisLeuAspLeuSerLeuGlySerThrGlnValLysAlaHisGlyGlnL
CCTACTTCCTCCACCTGGACCTGAGCCTCGGCTCCACCCAGGTCAAAGCCCACGGCCAGA
         C    T C  T       A       G          G         A
        Pro   Phe         His      Ala                  Lys

ysValAlaAspAlaLeuThrLeuAlaValGluHisLeuGluAspLeuProArgAlaLeuS
AGGTGGCCGACGCGCTGACCCTCGCCGTGGAGCACCTGGAAGACCTGCCCCGCGCCCTGT   600
      G            T          GC        C        TG
     Gly                     Gly      Asp         Gly

erAlaLeuArgHisArgHisValArgGluLeuArgValAspProAlaSerPheGln
CGGCTCTGCGCCACCGGCACGTTCGCGAGCTGCGAGTGGACCCCGCCAGCTTCCAGGTGA
  AA    A  G   T     CA A A       C          T  A    A
  Asn   SerAspLeu   AlaHisLys               ValAsn   Lys

GCCCGCGGGCCGGGGCGGGACCGCTGCTCCCAGCAGGGACGCGGCGGGGACGCACGGACC   720

                            LeuLeuGlyHisCysLeuLeuValThrProAl
CCGGGTGGGACGACCCCTCTCTCCGCAGCTCCTGGGCCACTGCCTGCTGGTGACCCCCGC
                              T   A T  T          TCC  TTG
                                  Ser            Ser   Leu

aArgHisPheProGlyAspPheSerProThrLeuHisAlaSerLeuValLysPheLeuSe
CCGGCACTTCCCCGGGGACTTCAGCCCTACCCTGCACGCCTCGCTGGTCAAGTTCCTGAG   840
 GTC   C     AAC  T    C    G  G C        C    A       T
 Val   Leu   Asn      Thr   AlaVal            Asp

rHisValIleSerAlaLeuAlaSerAspCysArg***
CCACGTGATCTCGGCGCTGGCCTCCGACTGTCGCTAAACCCGGGCTCGGTGGGTGCGGGG
 AGT    G A C T    A     A A AC  T
 Ser   SerThrVal   Thr   LysTyr

CCCCCCGGCTGCCCCTCCCGCGAGGTTCCAGCGTCGGAGTAAAGTCCCAAAAGCAAGCCC   960
GGTGCGCGTGAGCGTGTTCTCTCCGGCTCGGCGGGTGGGCGTCTGCCCCCCAGAGGAAGA
GAGGGGTCTGGGAGAGCCTGAGCCACAGCCGTCACTGTCACATCCGGGCACCCTGGGTGG
TCGAGGGAGAGGGTTCAGGTCGCCCCCACCCCTGGGGCCTGAGGCGAGTTTGGGAAGGCGG 1141
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Fig. 1 Nucleotide sequence of the sense strand of horse $\psi\alpha$ gene with (above) the translated protein sequence. Below the main sequence are the nucleotide and amino-acid changes that characterize the coding regions of the horse $\alpha 1$ gene and its expressed globin. A 1.5-kb *Mst*II fragment of plasmid pGG2 (ref. 3), containing the entire $\psi\alpha$ gene, was digested with *Hae*III, and a library of the resulting fragments constructed in the sequencing vector pGV403. DNA from size-selected subclones was sequenced on both strands by the method of Maxam and Gilbert[9] and aligned and overlapped with a partial sequence previously obtained[3] by sequencing from known starting points on the restriction map of the $\psi\alpha$ gene region.

Table 2 Important functional residues in α globin that are replaced in putative $\psi\alpha$ or $\theta 1$ protein

Animal	Residue	Substitution	Predicted consequence to structure
HBO	B6(25)	Gly → Ala or Thr	Glycine B6 makes a short contact with Gly E8. Insertion of a side chain forces helices B and E apart
BO	B13(32)	Met → Thr	Met B13 is an essential haem contact
HBO	C3(38)	Thr → Ser or Ala	Thr C3 is at the $\alpha_1\beta_2$ contact and is essential for the T↔R switch
H	C5(40)	Lys → Thr	Lys C5 is essential for the stability of the T-structure
H	F5(84)	Ser → Arg	Ser F5 is essential for the stability of the tertiary structure. There is no room for an Arg
H	F7(86)	Leu → Arg	Leu F7 is an essential haem contact
HO	G4(97)	Asn → Ser	Asn G4 is an essential haem contact
B	G6(99)	Lys → Pro	Proline would disrupt the G-helix
H	G16(109)	Leu → Pro	Proline would disrupt the G-helix
H	HC2(140)	Tyr → Cys	Tyr HC2 is essential for the stability of the tertiary structure

H, horse; B, olive baboon; O, orang-utan.

orang-utan α versus $\psi\alpha(\theta 1)$ comparison suggests a divergence time of ~250 Myr, assuming that $\psi\alpha$ and $\theta 1$ genes have been functional globin genes throughout this period. On the other hand, the number of silent site changes are roughly the same in each of these comparisons, though not reaching values generally considered to be saturated through multiple substitutions[6,7]. Although the discrepancy between replacement and silent substitution rates might be explained by the inherent uncertainties in the calculations of evolutionary divergence times, another possibility could be that the assumption that the $\psi\alpha$ and $\theta 1$ are globin genes is incorrect. Perhaps for much of the time since divergence from α, the $\psi\alpha$ and $\theta 1$ genes were rapidly accumulating amino acid replacement changes as they evolved to fulfil a new function. Their divergence could thus have occurred much more recently than the straightforward $\alpha 1$-$\theta 1$ ($\psi\alpha$) replacement site comparison suggests.

The predicted amino-acid sequence of the horse $\psi\alpha$ protein suggests that this possibility may need further investigation. Of the 53 changes in the $\psi\alpha$ protein sequence compared with horse α-globin, at least eight are in key structural regions and would severely impair the stability of a globin subunit, haem binding, or the R↔T structural transition that accompanies oxygenation and deoxygenation (Table 2). These considerations suggest that the horse $\psi\alpha$ protein, if it exists, is unlikely to function successfully as a haemoglobin subunit. As the primate $\theta 1$ protein sequences have some of these inactivating substitutions, as well as others, there must also be some doubt about their functional properties.

A search for transcripts in various tissues could help to clarify doubts about the transcriptional competence of the $\psi\alpha$ and $\theta 1$ genes[8], but will not provide much insight into their function. It is worth noting that humans completely lacking the $\theta 1$ gene have been born live[10], suggesting that if the $\theta 1/\psi\alpha$ genes have a function it is not one vital to fetal survival.

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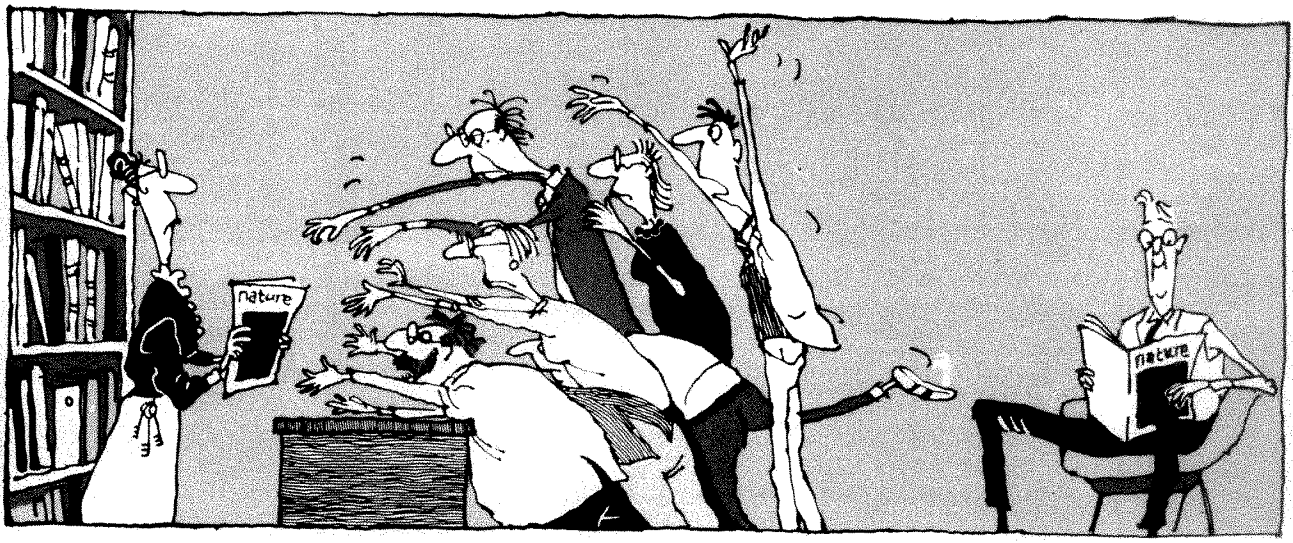

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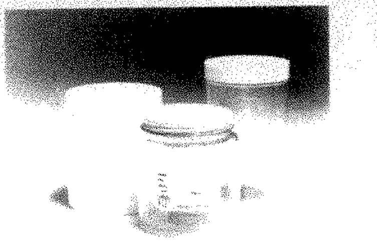

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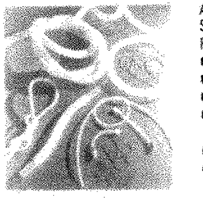

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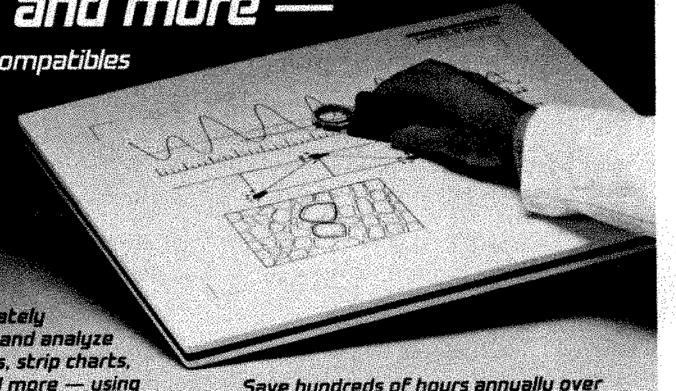

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Reader Service No.4

Plastic disposables in the laboratory

R. Conway and T. Chaplin

Plastic disposables have become essential equipment in most medical and industrial laboratories. The benefits they offer in cost, sterility and safety suggest that this trend is likely to continue.

THE large volume of samples generated by the increase in testing and analysis work in modern laboratories has created an increased demand for disposables. The glass 'laboratory durables' used for many years had a guaranteed life expectancy of ten years, and often lasted for fifty years or more. Today's plastic laboratory disposables, in contrast, are designed to be used once and then be thrown away.

By the time plastic Petri dishes and other early laboratory disposables began to appear on the market in the early nineteen fifties, the concept of disposability was already well accepted. It was the arrival of auto-analysis equipment designed specifically for use with plastic or glass disposable 'accessories', however, which started the current era of laboratory disposables.

The properties of early plastics were very limited. While some were flexible enough to be formed into a variety of shapes, they lacked heat resistance and clarity, and this restricted their potential applications. The development of more sophisticated tools and machinery for manufacturing plastics brought the production of plastics from a primitive chemical process to an industry of high precision engineering, with the capability to design and mould accessories to almost any specification. Advances in plastics technology have now created a new generation of disposables with better resilience, heat resistance, clarity and surface properties that compete with traditional disposables made of glass. The range of plastic disposables available today includes such basic items as Pasteur pipettes and test tubes, as well as precision pipettes and centrifuge tubes that can withstand up to 8,000 RCF.

Composition

The majority of plastic disposables are moulded or extruded from polystyrene, polypropylene and polyethylene (Table 1). Polypropylene differs from polystyrene and polyethylene because it is autoclavable and has the ability to withstand temperatures of up to 135 °C. Polypropylene and high density polyethylene can both withstand chemical sterilization with substances such as benzalkonium chloride, formalin and ethanol. Although polystyrene cannot stand up to extreme heat and strong chemicals, it has a high level of optical clarity.

Another new plastic, polycarbonate, is extremely resilient and has excellent optical clarity. Polycarbonate is classified as an engineering thermoplastic, because of its toughness, exceptional clarity and high heat resistance. It is made in an interfacial process by a reaction between bisphenol A and carbonyl chloride. Characteristics of this plastic include a heat deflection temperature of 130 °C and 87–91 per cent light transmission, and its impact properties make it ideal for centrifuge tubes.

One of the latest materials to be developed is very low density polyethylene (VLDPE) which can be drawn into thin film and does not tear easily. This new class of linear polyethylene polymer has a density range of 0.890–0.915 g cm^{-3}. VLDPE has greater flexibility than the other low density linear polyethylenes — such as the lower strength material EVA and plasticized PVC — but does not provide their toughness and broad operating temperature ranges. VLDPE can be used to produce thin films as low as 0.2 mm in thickness, and is suitable for applications requiring a soft, flexible material for disposables such as gloves and health-care products.

Table 1 Physical properties of plastic disposables

	Polystyrene	Polypropylene	High density polyethylene
Autoclavable	No	Yes	No
Brittle temperature	0 °C	0 °C	− 100 °C
Chemical sterilization*	No	Yes	Yes
Dry heat sterilization at 180 °C	No	No	No
Gas sterilization, ethylene oxide	Yes	Yes	Yes
Heat distortion temperature	70 °C	135 °C	121 °C
Specific gravity	1.040	0.903	0.944
Transparency	Clear	Translucent	Opaque

*Benzalkonium chloride, formalin, ethanol.

Precision

Disposables for liquid measuring and delivery, such as pipettes, require a high degree of precision in manufacturing to ensure that they are made to exact dimensions and will dispense exact volumes. Accordingly, precision plastics are made from high-quality components with specific constant properties.

The production of plastic disposables is backed up by strict quality control procedures. During manufacturing, pipette tips are subject to eleven different quality control procedures, and are screened according to uniformity of orifice geometry, cleanliness of hydrophilic surfaces, appropriate length and colour, and zero ovality. They are also tested for excess plastic residue, known as flash.

Plastics are able to deliver the precision required and still retain their benefits of low cost and disposability. A typical example is the micropipettor, where precision engineering is used to design the non-disposable plunger unit, while contact with liquids is restricted to the disposable pipette tip. Tips are usually made of polypropylene, which can be polished to a high gloss to avoid retention of liquid which might prevent a precise delivery.

The latest techniques in plastic manufacturing are producing disposables with specific surface properties. This type of disposable is very useful in tissue culture, where the surfaces of culture dishes must be specially treated to encourage the growth of a uniform monolayer of cells. The treatment of plastics has also produced multi-well plates which allow antigens to bind to their surface, making possible new immunological techniques such as ELISA and RIA.

Limitations

The applications of plastic disposables are limited by their moderate resistance to heat and chemicals. Although plastic tubing, for example, can be used with a strong base such as concentrated sodium hydroxide or a strong acid such as concentrated hydrochloric acid, it is not recommended for use with chemicals such as benzaldehyde, ethylene dichloride, acetic acid, ethers, concentrated sulphuric acid, lacquer solvents and perchloric acid. The manufacturers of plastic disposables provide safety guidelines for the storage and use of chemicals in conjunction with plastic products to prevent accidents arising from misuse.

The future in new plastics technology lies in the development of disposables to meet the changing requirements of the modern laboratory. The cost of raw materials and the introduction of stronger polymers will be determining factors in the future of disposables. □

Robin Conway and Tony Chaplin are at Elkay Laboratory Products Ltd, Unit 2, Crockford Lane, Basingstoke, Hants RG24 0NA, UK.

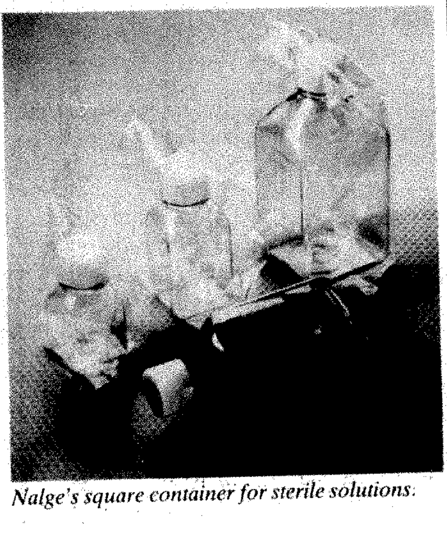
Disposables that make the grade

This week's feature on plastics and other disposables for laboratory use ends with a preview of several products to be exhibited next week at the American Society of Human Genetics meeting in San Diego, California.

FISHER now sells **microtome blades** in a hand-sized dispenser (*Reader Service No. 101*). The $60 (US) dispenser comes with 60 stainless-steel blades measuring 0.498 in. wide by 3.000 in. long, that are compatible with AO blade-holders. The blades' 0.012 in. cutting edge is coated with PTFE, to allow tissue sections to slide off the blade without distortion and "chattering". The blade dispenser is designed for safety: the transparent top permits viewing of the remaining stock, and used blades are placed in the bottom compartment. The package can be disposed of as a unit after the blade supply is depleted.

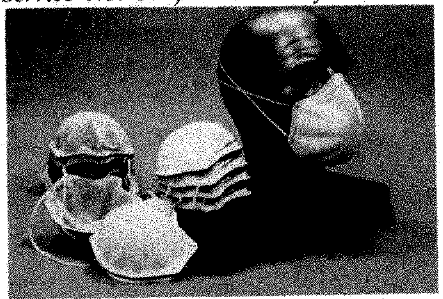
The **1986/1987 catalogue** from Evergreen Scientific contains a full range of disposables for the laboratory or clinic (*Reader Service No. 102*). Available free from the company, the 64-page catalogue covers filters, pipettes and tips, tubes and caps, biohazard bags, vials, cuvettes, chromatography columns, and specimen containers and mailers. New in this catalogue are hospital supplies such as patient admission kits, pathology specimen containers, a sputum collection kit, and histology supplies. Evergreen offers free samples of many of its products.

Sterile cell culture media, serum and other biological solutions are just right for Nalge's **sterile disposable square bottles** (*Reader Service No. 103*). The polystyrene bottles have a deep polypropylene closure and a narrow mouth to facilitate aseptic techniques. The square design gives the bottles stability, and makes it easier to pack them into refrigerators and freezers, or line them up under a lab hood. The bottles are radiation-sterilized and packaged in plastic, and have molded-in graduations for easy measuring. The bottles are available in 125, 250, 500 and 1,000 ml sizes, and are sold 48 and 24 to a case for $48–72 (US).



Nalge's square container for sterile solutions.

The Super Chemisorb **masks** from Safelab Systems Ltd are now available in a more anatomically-moulded shape (*Reader Service No. 104*). Safelab says the new design fits better, to give greater protection from noxious fumes, mists, dust and smoke to the wearer. The Chemisorb masks have activated charcoal filter layers that absorb aromatic and aliphatic hydrocarbons, sulphur and nitrogen compounds, halogens, acids, alcohols, esters and ethers, and aldehydes and ketones. The masks also have a BS-6016 Type 2 particle filter to keep out particulate contaminants. The low breathing resistance of the masks permits normal speech. The masks come 10 to a box for £25 (UK) or 200 to a carton for £450 (UK).

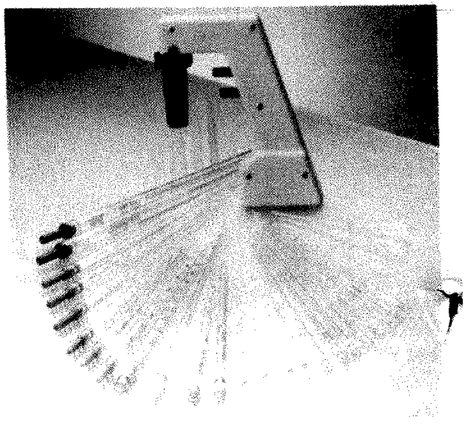


Masks to guard against vapours and particles.

For **cleaning up laboratory spills**, Matarah Industries has an on-the-spot solution, called Labdab (*Reader Service No. 105*). Labdabs are circular absorbent blotters backed with chemical-resistant polyester and a pinch-tab for handling. The Labdabs come in 3, 6, 9 and 12 in. diameter sizes. Matarah says they absorb up to 15 times their weight in liquid: the 3 in. Labdab absorbs up to 10 ml. The $24–27 (US) Labdabs come 15 to a pack in a dispenser that can be mounted on a handy place near the lab bench.

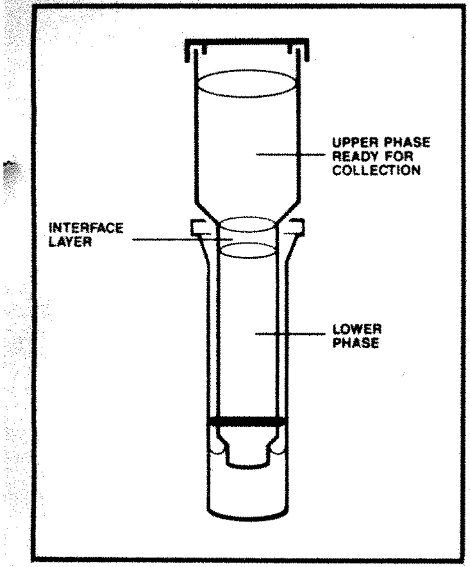
World Precision Instruments offers **disposable ion-selective electrodes**, to avoid contaminating one solution with residue from a previous one (*Reader Service No. 106*). The Kwik-Tip disposable tips are 3 mm in diameter plastic tubes covered at one end with a membrane sensitive to a specific ion. To use, the tubes are filled with an appropriate electrolyte, inserted into the reusable holder and connected to a *p*H meter. WPI says the tips can be replaced in less than a minute, to measure calcium, potassium, tetramethyl ammonium, sodium and chloride ions in solution. The Kwik reusable holder comes with a BNC cable and an electrolyte filling syringe for $50 (US), and a choice of one of the five Tip electrode kits costs $50 (US). WPI recommends its MERE 1 reference electrode for use with the Kwik-Tip system.

Bulk packs of **disposable pipettes** are available from Sterilin (*Reader Service No. 107*). They are manufactured from polystyrene, and are supplied plugged and sterile, in open-ended or tipped versions. The tipped varieties are made from a single piece of polystyrene, and do not have fluid-trapping shoulders near the tip. The open-ended versions are suitable for fast delivery of volumes, or for pipetting viscous fluids. The pipettes are available with or without suction adapters, and are colour-coded according to size. They come in 1, 2 and 5 ml sizes, in packs of ten.



Sterilin's rainbow of disposable pipettes.

Xydex has a disposable product for **liquid extractions** — the PhenX (*Reader Service No. 108*). The PhenX can be used for phenol extractions of DNA from lysates, plasmid preps and agarose gels, and for the purification of lipids from serum, plasma samples or cell membranes. The $42 (US) PhenX kit consists of 48 sets of three sample tubes and a special plunger. After mixing and centrifugation of the extraction phases, the plunger is pressed into the sample tube, forcing the aqueous layer into the plunger lumen and upper compartment. The separated aqueous phase can then be poured or pipetted into another container, leaving the interface layer and organic phases

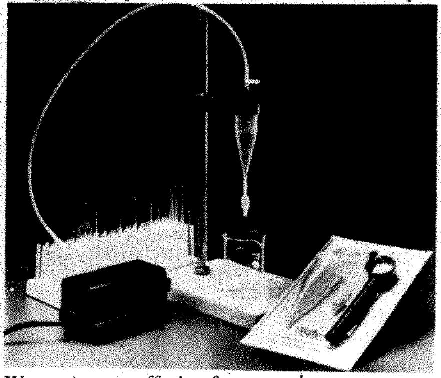


For phenol extractions, and more.

behind. Xydex says the PhenX performs microextractions of 3 ml samples with recovery rates of over 40 per cent.

Elkay is offering free **disposable cuvette** sample packs to laboratories willing to evaluate the six different cuvettes manufactured by the company (*Reader Service No. 109*). The cuvettes are moulded in UV inhibitorless polystyrene or acrylic copolymer, and are capable of averaging 70 per cent light transmittance at 340 nm, says Elkay. The cuvettes are supplied in particle-free shrink-wrapped reusable trays. The six cuvettes in the pack include round and square types, made from polystyrene and acrylic.

A disposable **solid-phase extraction system** for sample preparation is available from Waters, a division of Millipore (*Reader Service No. 110*). The $5.95 (US) Sep-Pak system kit contains sample reservoirs, reservoir supports, C_{18} cartridges and an air pressure pump. The C_{18} cartridges provide sample preparation in a variety of chemistries, and connect to the bottom fitting of the sample reservoir. Solution volumes ranging from 0.5 ml to 30 ml can be extracted and filtered using the Sep-Pak. Waters is offering free samples of the Sep-Pak system to researchers who would like to try it out.

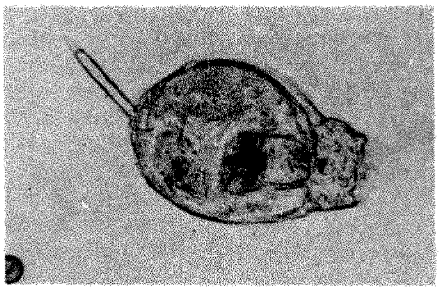


Waters is now offering free samples.

Human genetics

Applied Biosystems will be exhibiting its line of instruments for the synthesis, isolation, purification and sequencing of nucleic acids and peptides in booths 233 and 235. Sure to be on display is the model 430A **peptide synthesizer** (*Reader Service No. 111*). Fmoc chemistry is now available for the $79,500–87,500 (US) system, complete with synthesis protocols and a complete range of "plug-in" chemicals. The system can be adapted for t-Boc chemistry — recently introduced with an alternative to HF — by simply changing the floppy disk and loading the relevant chemicals. The model 430A synthesizer can be used with Applied Biosystems' model 151A peptide purification system for a complete system for peptide synthesis, from chain assembly to cleavage and final purification.

Nikon has a new ×60 **phase contrast dry objective lens** that extends the magnification range of the Diaphot binocular laboratory microscope to ×900, using an ×15 eyepiece (*Reader Service No. 112*). The lens extends the Diaphot's resolution over a long working distance of up to 3.07 mm. Cover glass thickness compensation is included in the Diaphot microscope, and there is no need for a special turret condenser. Nikon also has two new phase plan achromatic objectives, with magnifications of ×20 and ×40. The ×20 has a working distance of up to 6.0 mm, and the ×40's working distance ranges from 5.08 mm to 6.48 mm, according to cover glass thickness. Nikon will be displaying a full range of its microscopes and objectives in booths 135 and 137.



Shot of a rotifer, 300 μm in length, taken with Nikon's Diaphot with the new × 60 lens.

For the early detection and monitoring of **prostatic cancer**, Hybritech has the Tandem-R PSA immunoradiometric assay (*Reader Service No. 113*). The assay measures serum prostate specific antigen (PSA) — shown to be a more sensitive marker for prostatic cancer than prostatic acid phosphatase (PAP) — quantitatively. Hybritech says the assay achieves a minimal detectable PSA concentration of less than 0.15 ng ml^{-1}, and is linear to 100 ng ml^{-1}. Clinical trials at several major cancer research centres, including Johns Hopkins Hospital and the Memorial Sloan-Kettering Institute, demonstrated that 95.7 per cent of patients had continually decreasing levels of PSA after successful therapy, as shown by the Tandem-R PSA assay. The assay also indicated tumour recurrence: 94.6 per cent of patients exhibited PSA elevations prior to and during the onset of progressive or recurrent prostate cancer. The Tandem-R PSA assays cost $590 (US) for an antibody and a substrate set for 100 determinations. Hybritech will be in booth 230.

Hoefer Scientific has a bulletin that describes a **technique for DNA microassays** (*Reader Service No. 114*). Small amounts of DNA are difficult to quantify especially in the presence of RNA or protein. Hoefer's Technical Bulletin Number 119 explains how to determine nanogram to milligram amounts of DNA in dilute samples, using the fluorochrome Hoechst 33258. The technique can be used with Hoefer's IKO 100 DNA minifluorometer, or other fluorometer. Hoefer will be exhibiting its line of instruments for gel electrophoresis in booth 236.

To help valuable DNA restriction and modifying enzymes keep their cool on the way from the freezer to the lab bench, Stratagene has a **portable enzyme storage box** called the StrataCooler (*Reader Service No. 115*). The $165 (US) StrataCooler holds up to 24 standard microcentrifuge tubes, and keeps them at a temperature of −20 °C. The box is constructed of durable acrylic filled with a special freezing agent that maintains the temperature inside at less than −15 °C for over two hours on the bench, according to Stratagene. Stratagene will be displaying its wares in booth 127. ☐

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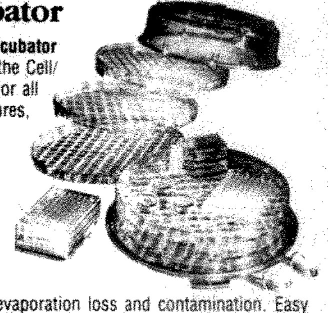

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Reader Service No.174

Less unemployment, or skills shortage

Richard Pearson

Population changes in the United Kingdom during the next decade may help solve one problem but will create others which are difficult to predict.

We are now entering an era of sharp demographic change. Across most of the non-catholic Western world (though not in Japan), the number of 16–18-year-olds in the population will decline rapidly over the next decade as a result of the slump in births in the 1960s. In the United States there is a 23% decline between 1908 and 1994, while in Germany the decline is a massive 44%. In the United Kingdom the downturn will be 26% over this period, with most of the decline during the early 1990s (*Nature* **326**, 528; 1987).

Is this decline going to be the answer to the United Kingdom's long-term problem of high youth unemployment? In areas of high unemployment perhaps, but the consequences for some employers, course organizers and training organizations will be profound. Already the health service cannot recruit enough school leavers into nursing, while many of the government's Youth Training Schemes, set up to improve the vocational skills of young people, have empty places because better paid full-time jobs attract leavers in many areas of the South East. These problems are being experienced when the number of school leavers is near its peak and the decline has barely begun.

Table 1 Projections of number of 18-year-olds in the United Kingdom by social class (thousands)

Year	Social class						Total
	I	II	IIIN	IIIM	IV	V	
1980	56	186	93	329	165	52	881
1985	66	202	93	343	145	46	893
1990	70	213	85	311	96	30	801
1995	63	183	62	215	72	19	613
2000	61	213	67	242	73	25	681

Key: I, professional; II, intermediate; IIIN, skilled non-manual; IIIM, skilled manual; IV, semi-skilled; V, unskilled. Source: AUT

Major differences in the birth rates between social classes — with differing occupational aspirations and qualifications — are an added complication. Because the decline in the birth rate among the higher social classes has been less severe (Table 1), the decline in better qualified leavers will also be less severe. So the reduction in demand for places in higher education will be less than crude numbers suggest. Unfortunately, while forecasts of school leavers with differing qualification profiles are available up to the early 1990s (Table 2), changes in the exam structure make longer term forecasts extremely speculative.

Table 2 School leavers available for employment (1979/80–1992/93)

Examination achievement	Percentage decline 1979/80–1992/93
A levels	– 21 %
5 or more 'good' O levels*	– 34 %
1–4 'good' O levels	– 40 %
Other O level/CSE	– 35 %
No graded results	– 58 %
Total	– 40 %

* 'Good' O levels are O level passes graded A to C and CSE grade 1. Forecasts for 1987/8 and beyond are complicated by the existence of GCSE. Source: DES/IMS.

What then are the most important implications of these trends, and which sectors need to adopt new policies to cope? Clearly, at the top end of the qualification profile competition for recruits is going to become ever more fierce.

The banks, for example, who take many thousands of well qualified school leavers each year, will be in direct competition with technology courses in higher education for the school leaver with two A levels. A factor which may make things worse for organizers of technology courses is a possible replication in the United Kingdom of the trend in the United States where students are turning away from engineering and technology (*Nature* **329**, 90; 1987).

Further down the qualification profile the health service is going to face a major challenge. Even now the health service cannot recruit enough nurses, and with the potential pool of qualified candidates shrinking the prospects are poor.

What can be done to mitigate the unwanted side effects of the post-baby-boom era? A first step is for an organization to be aware of its dependence on school leavers, and numbers are not the sole guide. An employer in the South East seeking five trainee technicians with science O levels may face far greater difficulty than a high quality Youth Training Scheme in the North West seeking 50 trainees. It is line managers and department heads who also have to be aware of these changes and not try to maintain rigid, historic recruitment criteria in the face of this changed external environment. Innovation in recruitment, training and employment will be needed and has to be a joint responsibility between them and the recruiters.

Next is the need to review the reasons that lay behind the decision to recruit a specific category of school leaver. For example, are five O levels really necessary for a given job? It may be that entry standards have been raised in response to the easier recruitment environment of the past few years. Qualifications are also often seen as the only screen for intelligence and competence; the Youth Training Scheme has now demonstrated that alternative forms of experience can be just as relevant. Likewise is a physics or mathematics A level really necessary, what about the combined science qualifications or an aptitude or numeracy test?

Recruitment can also be redirected to other groups; one new source of skills that has been opened up by the recession and the increasing rate of change in the labour market is that of the mature (re)entrant or individual seeking retraining. The potential here is enormous and these people can often bring new insights and additional skills to a job. The obvious source here is women returning after child rearing. Finally recruitment levels may not need to remain at traditionally high levels. A reassessment of job content, induction training, selection, job previewing and type of entrant can all contribute to lowering wastage during training and early careers, thus reducing the need for replacements as well as improving job satisfaction for the individual.

The demographic downturn is going to provide both opportunities and problems. While reductions in youth unemployment will of course be welcome, many recruiters for both jobs and training courses will have to think long and hard about innovation and overturning tradition if they are to continue to meet their employment needs during the next decade and beyond. □

Richard Pearson is at the Institute of Manpower Studies, Mantell Building, University of Sussex, Brighton BN1 9RF, UK.

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Applicants should hold either F.I.M.L.S., HND or BSc qualification in a biological science. Salary for this post will be in the range of £8-£9,000 pa depending on qualifications and experience. (Ref. 2534).

RESEARCH SCIENTIST – Metabolism and Pharmacokinetics

The Department of Metabolism and Pharmacokinetics examines the disposition of foreign compounds (drugs, veterinary products, pesticides, etc.) in animals and man. The main duties associated with this vacancy will involve participation in the biological and particularly the analytical aspects of drug metabolism studies. A special interest in chromatographic methods would be an advantage.

The appointment would suit a recent Honours Graduate Chemist, Biochemist or Pharmacologist, perhaps with some industrial experience, preferably of TLC, HPLC and radiochemicals.

Initial salary for this position will be in the range £7-£8,000 p.a.

In addition the Company offers a wide range of benefits including free life assurance cover, participation in bonus scheme arrangements, membership of the Company's excellent pension plan and generous assistance with relocation expenses, if appropriate.

Full details in writing should be forwarded **as soon as possible** to the **Personnel Department, Inveresk Research International Limited, Inveresk Gate, Musselburgh EH21 7UB,** quoting the relevant reference number. (4169)A

IRI INVERESK RESEARCH INTERNATIONAL

PRINCIPAL SCIENTIST/ PLANT MOLECULAR BIOLOGY

The position is to manage an Independent research team in an area of plant genetic engineering with the objective of defining and creating new and novel business opportunities for the application of this technology. In addition, the individual will oversee the execution of confidential research contracts in this same area. Duties include: 1, Develop novel methods in plants to target proteins to the chloroplast. 2, Develop techniques for over expression of foreign genes in plants by using promoter fragments from photosynthetic genes. 3, Evaluate factors within strongly expressed plant genes contributing to high levels of expression. 4, Oversee IBC guidelines. Ph.D. or equivalent in Biology or Biochemistry. 4+ years required experience in plant genetic engineering. Research experience must include molecular analysis of plant genes, overexpression of foreign genes in plants using photosynthetic gene promoters, sequence analysis & characterization of plant gene expression levels, and generation of transformed plants. Also required working knowledge of IBC guidelines and must have record of publications in major scientific journals. $45,000/year; 40 hrs/week. If offered employment, must have legal right to work. Clip ad & send to:

6701 SAN PABLO AVENUE, OAKLAND, CALIFORNIA 94608

Attn: K. Lang, not later than October 21, 1987. (NW596)A

PLANT MOLECULAR BIOLOGIST

The Department of Biochemistry and the Agricultural Experiment Station of the University of California, Riverside, are seeking a new faculty member in the area of plant molecular biology. The position is to be filled at the assistant (tenure-track), associate or full professor level, commensurate with the experience of the successful candidate. The new faculty member will hold 11-month appointments jointly in the College of Natural and Agricultural Sciences and the Agricultural Experimental Station. Requirements for this position include a background in plant biochemistry and molecular biology, and a demonstrated record of productivity in the general area of molecular biological techniques as applied to plants. Both the strength of the existing research record and the potential for developing a strong, innovative research program will be considered for each candidate. Young scientists are encouraged to apply, as are minority and women candidates. Applicants should send curriculum vitae, and arrange to have at least three letters of recommendation sent to: **Justin K. M. Roberts, Search Committee Chairman, Department of Biochemistry, University of California, Riverside, CA 92521.** Application deadline is 31 October 1987 or later if suitable candidates have not been selected.

The University of California is an Equal Opportunity/Affirmative Action Employer. (NW603)A

UNIVERSITY OF OXFORD
INSTITUTE OF MOLECULAR MEDICINE

POST-DOCTORAL RESEARCH FELLOW

An experienced experimental electrophysiologist is required to extend the neurophysiological programme with a Neuroscience group investigating the molecular basis of immunological and inherited disorders of ion channel function (acetylcholine receptors, calcium channels) at the neuromuscular junction.

The appointment would be for 3 years in the first instance, and is funded by the Muscular Dystrophy Group of Great Britain. *Salary according to age and experience.* The post will be available from March 1st, 1988. Applications including full cv, publication list and addresses of 2 referees should be sent by November 15th to **Professor J. Newsom-Davis, Department of Clinical Neurology, University of Oxford, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE** (Tel: 0865 727347) from whom further information may be obtained. (4171)A

UNIVERSITY OF ILLINOIS, CHICAGO
COLLEGE OF MEDICINE

ASSISTANT/ASSOCIATE PROFESSOR

The Department of Biological Chemistry is re-opening its search for a tenure track Assistant/Associate Professor position effective academic year 1988 or later. The candidate is expected to establish an independent research program in the area of biochemistry and biophysics of signal transduction with specific emphasis on molecular mechanisms. The Department is research oriented with a commitment to graduate and biomedical education. Applicants should provide a curriculum vitae, the names of three people for references, and a statement of research interests and plans by December 1, 1987. Send applications to: **Dr James C. Vary, Department of Biological Chemistry, University of Illinois at Chicago, College of Medicine, 1853 W. Polk Street, Chicago, IL 60612.**

The University of Illinois is an Affirmative Action/Equal Opportunity Employer. (NW583)A

Research Leaders in Virology

Middlesex c£20K

To meet the aims of the Virology Unit at Glaxo Group Research, two new positions of Research Leader have been created. One will have the responsibility of initiating further research programmes on the biochemistry and molecular biology of herpes viruses, and the other on influenza viruses, with the long term aim of defining novel targets for viral therapy. Each Research Leader will also be expected to establish and manage a group of 6-10 scientists and technicians involved in this work.

These are senior positions for which you should possess a PhD and postdoctoral experience of the molecular biology or biochemistry of either herpes viruses or influenza viruses, with a proven record of research and innovation in one of these areas.

The salary quoted above is given as a guideline only: the final package will include an annually reviewed basic salary plus profit-related bonus, a non-contributory pension and full help with relocation where appropriate.

These new posts reflect the new phase of growth which Glaxo Group Research is entering following a decision by Glaxo Holdings, our parent Company, to invest substantial additional resources in the research and development of new and existing ethical pharmaceuticals.

To be considered for these posts please send a full CV or telephone for an application form to Ted Smith, Senior Personnel Officer, Glaxo Group Research Limited, Greenford Road, Greenford, Middlesex UB6 0HE. Tel: 01-422 3434, ext. 2934, quoting reference number 104.

(4231)A

Glaxo Group Research Ltd.

CALIFORNIA INSTITUTE OF TECHNOLOGY

Parallel Computing Project Team Leader for the L3 High Energy Physics Experiment

The L3 Collaboration will install the L3 Parallel Integrated Computing System (LEPICS) at CERN, Geneva in October, 1987. LEPICS will be the primary center for the analysis of L3 data from the LEP electron–positron collider, and the focus of the worldwide LEP3NET network by 1989. The system will consist of an IBM 3090 mainframe coupled to attached processors at channel speeds. An L3–IBM joint project has been set up to make parallel processing work effectively, and largely automatically, in a computer center environment. During 1988 and early 1989 software development is planned through which LEPICS will become a powerful and highly expandable parallel computing center for high energy physics.

The Caltech group within the L3 Collaboration is responsible for offline computing for the experiment, including the LEPICS Parallel Processing Project. The group is now seeking a team leader to work full-time on the project at CERN. The team leader may be either a high energy physicist, or a computing specialist. Candidates should have a Ph.D. or extensive experience in computing. An understanding of IBM VM/CMS is an advantage. The ability and desire to take major resonsibility for a vital, highly visible computing project is essential.

Applicants should submit a curriculum vitae, bibliography and a description of research experience and interests. Anyone interested in this position is strongly encouraged to request further information from:

Dr. Richard P. Mount
EP Division, CERN
1211 Geneva 23, Switzerland
(41) 22 – 83 – 6366
MOUNT@CERNVM

Prof. Harvey B. Newman
256–48, HEP, CALTECH
Pasadena, California 91125
(818) 356-6656
NEWMAN@CITHEX
NEWMAN@cithex,caltech.edu

Caltech is an Equal Opportunity/Affirmative Action Employer. Women and minorities are encouraged to apply. (NW597)A

POSITION ANNOUNCEMENT

ENTOMOLOGIST (ORNAMENTAL HORTICULTURE)

(condensed for advertisement)

The Department of Entomology, University of California, Davis, announces a tenure-track faculty position in applied entomology (11 mo. plus 1 mo. paid vacation) at the Assistant or Associate Professor/Entomologist level. Ph.D. in Entomology or related discipline required. Appointee must become involved in undergraduate and graduate instruction and advising in entomology, supervise graduate students, and participate in the Graduate Program in Plant Protection and Pest Management. Appointee will be expected to develop a mission-oriented research program directed toward management or arthropod pests of ornamental plants. Fundamental research is also encouraged. Applicants should submit letter of application, curriculum vitae, summary of teaching and research goals, list and reprints of publications, and names and addresses of three referees to **Dr. L. E. Ehler, Chair of Search Committee, Department of Entomology, University of California, Davis, California 95616,** no later than December 15, 1987.

THE UNIVERSITY OF CALIFORNIA IS AN AFFIRMATIVE ACTION/ EQUAL OPPORTUNITY EMPLOYER. (NW598)A

POST DOCTORAL FELLOW

To study the molecular mechanisms of immunodeficiency virus (HIV) infections using molecular biology techniques. Qualifications include a Ph.D. degree or the equivalent in molecular biology or biochemistry, with experience in tissue culture, cloning and gene sequencing preferred. Position available immediately with funding support for at least 2 years. Please send application, with curriculum vitae and 2 names for reference purposes (and quoting Post Doctoral position), to:

Dr. B.R.G. Williams/Dr. A.S. Lau
Division of Infectious Diseases
Hospital for Sick Children
555 University Avenue
Toronto
Ontario
Canada M5G IX8.

(W4453)A

Viral Immunologist

The Upjohn Company, a leader in worldwide research, manufacturing and marketing of pharmaceuticals, chemicals and agricultural products, is seeking a Ph.D. scientist to join a growing research team dedicated to the development of novel vaccines for diseases of veterinary interest.

Applicants will be expected to have specific expertise in the research technologies needed to define viral proteins which confer serologically and cellularly mediated protection. They must be able to conceptually and strategically advance our knowledge in the area of epitope immunology and immunopotentiation as it applies to the development of novel vaccines, both through individual projects, and through interaction with other scientists.

Kalamazoo is a medium-sized Southwestern Michigan community with immediate access to a mix of excellent cultural, educational and recreational pursuits, including numerous lakes and a four-season climate. Upjohn offers an excellent salary commensurate with experience and an outstanding benefits program. For confidential consideration, please call toll free 1/800/253-8600, ext. 3-6767. (inside Michigan call collect 616/323-6767) to request an employment application be sent to you. Please refer to Ad Number 21960-F when calling. An Equal Opportunity Employer M/F

(NW609)A

Upjohn

Our commitment to scientific excellence continues...

Manager-
Pharmaceutical Microbiology

Newly Created Post

Package from £25,000

Our client, Glaxo Group Research, is part of the world's fastest growing pharmaceutical company and is internationally renowned for the quality of its science. The company is in an exciting phase of expansion and development designed to ensure its continued success well into the future.

The 300-strong Pharmacy Division plays a key part in the Company's product development activities. In mid 1989 it will occupy new purpose-built facilities at Ware in Hertfordshire, which will be among the most advanced of their kind.

In anticipation of this move, the Company wishes to appoint an innovative applied microbiologist to establish and run a new Pharmaceutical Microbiology Unit of up to 15 staff to support its formulation and analytical development activities. The Unit's role will be to carry out microbiological studies to assure the sterility and longevity of products and the protection of the environment, and to conduct research into rapid methods of testing preservatives and indentifying isolates.

The successful candidate will report to the Director of Pharmaceutical Analysis and will define, with senior management, the detailed objectives, standards and priorities of the Unit, and recommend resources and equipment. In addition to day-to-day management of the Unit, a key responsibility will be to identify and investigate novel means of determining the microbiological quality of pharmaceutical products, and to develop these into reliable automated procedures. Close links with academic centres of excellence will be encouraged.

For this challenging senior position we are seeking a microbiologist with a proven record of innovative achievement in an R&D environment. This experience will have been gained within the pharmaceutical industry or similar, such as foods research, and will be complemented by competent managerial and leadership skills.

The post carries an excellent remuneration package, including non-contributory pension, substantial profit-related bonus, and generous relocation assistance will be provided where appropriate.

Please write in confidence together with a copy of your curriculum vitae, or 'phone if you would like to discuss the appointment before applying:

Talentmark
Leaders in Health Care Recruitment

James A. Edwards, Talentmark Limited,
King House, 5-11 Westbourne Grove,
London W2 4UA. Tel: 01-229 2266.

(4232)A

McGILL UNIVERSITY, FACULTY OF MEDICINE
DIVISION OF UROLOGY

ASSISTANT PROFESSOR

Applications are invited for a faculty position at the rank of assistant professor. The successful candidate must have a Ph.D. or M.D., be eligible to scholarship programs from granting agencies supporting biomedical research and have 2-3 years of postdoctoral research experience. The candidate is expected to develop an independent research program in bladder physiology and/or pharmacology. Forward an application letter, a curriculum vitae, a list of publications and the names of three referees to: **Dr Claude Gagnon, Director, Urology Research Laboratory, Royal Victoria Hospital, 687 Pine Ave. W., Room H6:46, Montreal, Quebec, Canada, H3A 1A1.** (NW568)A

POSTDOCTORAL POSITION

available immediately for NIH-funded study on the structure and function of the parasitophorous vacuole membrane of *Plasmodium falciparum*. Other ongoing research includes studies on: pH and Ca^{2+} homeostasis in *Plasmodium* and mechanisms of parasite invasion of erythrocytes using digitized video-intensified fluorescence microscopy; peptide hormones, secondary messengers, protein kinases of *Plasmodium*; and immunological aspects of malarial infections. Send curriculum vitae with names of three references to: **Dr Ross B. Mikkelsen, Radiobiology Division, New England Medical Center, Boston, MA 02111.** (NW584)A

RESEARCH TECHNICIAN

Sloan-Kettering Institute is actively engaged in a series of projects related to the study of diagnostic and treatment approaches for immunocompromised patients. An opportunity is available to join a biomedical research team, and participate in the implementation of experiments which focus on the HIV 1, HIV 2, and HTLV retrovirus strains. You will plan and perform experiments utilizing molecular and immuno techniques, to evaluate efficacy of related drug therapeutic approaches. BS/BA in Immunochemistry, Microbiology or Biochemistry required. Hands-on experience with Western Blotting, the ELISA assay, and cell culture techniques a must. Become a contributor to a highly relevant research effort. Send resume to: **E.L. Kleinert, Personnel Dept. #272, Memorial Sloan-Kettering Cancer Centre, 1275 York Ave., NYC 10021**. *An equal opportunity employer, m/f/h/v.* Where the exceptional is the rule. (NW582)A

MARIE CURIE RESEARCH INSTITUTE

GROUP LEADER

We seek a suitably qualified and energetic scientist to direct a well-supported major initiative concerned with the molecular biology of malignant melanoma. Topics to be investigated could include a search for causative genetic lesions in clinical material, an examination of the molecular basis of cellular transformation by U.V. light, a study of aberrant gene transcription, melanocyte growth regulation etc., etc. Close links with clinical units already exist. The appointee will be expected to co-ordinate and control the work of clinical research fellows, research assistants and visiting workers and to recruit post-docs.

The salary will be on the Lecturer or Senior Lecturer scale (currently £14,245 up to £23,355). For further information, telephone: **Dr Graham Currie on 0883-723055**. Applications (full c.v. and the names of two referees) to Marie Curie Research Institute, The Chart, Oxted, Surrey, RH8 0TL. (4170)A

SMITHSONIAN TROPICAL RESEARCH INSTITUTE

DEPUTY DIRECTOR

The Smithsonian Tropical Research Institute (STRI) invites applications and nominations for the position of Deputy Director which falls vacant due to the appointment of Dr James R. Karr as the Bailey Professor of Biology at Virginia Polytechnic Institute. STRI, located on the Isthmus of Panama, is a bureau of the Smithsonian Institution. The institute is devoted to basic research throughout the world's tropics and is responsible for custodianship of a biological reserve, the Barro Colorado Nature Monument, and for the operation of a number of other facilities on the Isthmus for the support of terrestrial and marine tropical research.

The Deputy Director reports to the Institute's Director and is responsible for assisting the Director with the development of policies and the management of the Institute. The Institute's principal responsibilities are to promote research and education in the field of ecology, evolution, behavior, anthropology and paleontology.

Candidates should possess a PhD, engaged in independent research in a field related to STRI's broad interests and should have experience appropriate to the effective management of an international research mission in a complex administrative environment. Knowledge of the Spanish language would be useful.

Applications should consist of a letter of interest, curriculum vitae, and names and telephone numbers of three references. Nominations with supporting statements are also welcomed. Replies should be submitted before December 15 to:

SEARCH COMMITTEE (DEPUTY DIRECTOR)
SMITHSONIAN TROPICAL RESEARCH INSTITUTE
APO MIAMI 34002-0011

The salary range for this position is $53,830—$72,500. In addition, allowances for housing and overseas living are paid. The starting date for this appointment is no later than 1 September 1988. STRI is an Equal Opportunity Employer and appointments can be made without regard for nationality. (NW537)A

UNIVERSITY OF BATH

School of Biological Sciences

POSTDOCTORAL RESEARCH OFFICER IN MOLECULAR BIOLOGY

Applications are invited for
postdoctoral position availab
immediately to work on t
molecular genetics of the fung
Metarhizium anisopliae. This po
has been created by the establis
ment of an AFRC Link Researc
Group involving the University
Bath and the Institute of Horticu
tural Research (formerly GCR
at Littlehampton. The successf
candidates will join a large grou
at Bath University working o
fungal pathogens of insects. Th
aim of this new initiative is
develop and utilize recombina
DNA techniques to investigate th
molecular basis of fungal path
genicity.

The appointment will initial
be for two years and three month
and applicants should preferab
have research experience of mole
cular biology. Informal enquiries
contact Dr John Clarkson or
Keith Charnley on 0225 826826.

Starting salary up to £11,015 pe
annum.

Application forms and furthe
details from the Personnel Office
University of Bath, Clavertor
Down, Bath BA2 7AY or phon
Bath 826826 ext. 5033 quoting ref
87/182. Closing date: 15.10.87.
(4198)A

PENNSTATE

University Park Campus

DEPARTMENT HEAD BIOLOGY

The Department of Biology, The Pennsylvania State University, seeks to fill the position of Head of the Department.

Applications and nominations of established scientists with a strong research record are invited; teaching and administrative experiences are desirable. The department has 27 faculty members at the University Park Campus and 33 at the Commonwealth Campuses. The department head must interact with a variety of departments and with intercollege programs in ecology, genetics, physiology and plant physiology. Preliminary inquiries encouraged.

Candidates should submit curriculum vitae, list of publications, and names, addresses and phone numbers of three references to: Professor Ronald A. Pursell, Chairman of Search Committee, Box 79, 211 Whitmore Laboratory, **The Pennsylvania State University,** University Park, PA 16802. Phone: (814) 865-9651. Review to begin in November; closing January 4, 1988 or until suitable candidate is found. (NW591)A

An Equal Opportunity/Affirmative Action Employer

BARTOL RESEARCH INSTITUTE

UNIVERSITY OF DELAWARE

FACULTY POSITIONS

The Bartol Research Institute invites applications for two tenure track positions as part of its five year growth plan. Level and salary depend on experience, but appointments at the assistant professor level are preferred. Preference in the current hiring will be given to the areas of astrophysics, solar-terrestrial and space physics, particle theory and cosmology, although appointments in other areas of current Bartol research activity are not precluded. There is a preference for one of the appointments to be an experimentalist. The Bartol Institute and the Department of Physics and Astronomy conduct a joint graduate program on the University campus. From its South Pole station Bartol carries out a number of observations including helioseismology, cosmic ray astrophysics and studies of the cosmic microwave background. The Institute also conducts a varied program of research on the University of Delaware 2 million volt Van de Graaff accelerator. Resumes and three letters of recommendation should be sent to the President, Bartol Research Institute, University of Delaware, Newark DE 19716, to arrive no later than November 1. The appointments have a nominal starting date of September 1, 1988.

Bartol is an equal opportunity employer
(NW403)A

PROGRAM IN MOLECULAR BIOLOGY & BIOTECHNOLOGY

The University of North Carolina at Chapel Hill

Tenure track faculty position in molecular biology: We are specifically seeking candidates who exploit the potential of *in vitro* mutagenesis to alter structure and function of proteins, the goals being the elucidation of the mechanisms of enzyme catalysis, protein-protein and protein-nucleic acid recognition and/or allosteric phenomena. The successful candidate will join a vigorous, developing focus of excellence in structure/function studies within the multidisciplinary Program in Molecular Biology & Biotechnology, which brings together a campus-wide array of resources to this effort. The tenure home will be within the Department of Chemistry, and faculty rank will be determined by the candidate's qualifications. Send curriculum vitae, the names of three references, and a research plan to:

Marshall H. Edgell, Director, Program in Molecular Biology & Biotechnology, The University of North Carolina at Chapel Hill, 402 Swing Building 217H, Chapel Hill, North Carolina 27514.

Affirmative Action/Equal Opportunity Employer. (NW564)A

Biotechnology Research and Development

Pandex, a division of Baxter Healthcare Corporation, is developing state of the art products for medical diagnostics.

We are expanding our R&D group and offer unique opportunities for creative Ph.D. level Scientists interested in positions that offer challenging opportunities for professional career growth.

We are seeking the following key individuals:

Nucleic Acid Chemist - Ph.D. with 0-3 years postdoctoral experience in DNA/RNA synthesis.

Molecular Biologist - Ph.D. with 2-3 years postdoctoral experience in DNA cloning and synthesis of DNA probes.

Assay Development Scientists - Ph.D. with 3-5 years postdoctoral or industrial experience in immunology, virology or microbiology with knowledge of immunoassay techniques.

Virologist - Ph.D. with 0-3 years postdoctoral experience or equivalent in retrovirology.

Microbiologist - Ph.D. with 0-3 years postdoctoral experience in the area of STD's.

As a division of a multi-billion dollar leader in the healthcare industry with research facilities located 30 miles north of Chicago, we offer a competitive starting salary and an excellent benefits program.

If you would like to be considered, please send your resume in confidence to: Anne McNicholl, **Personnel Director, Baxter Healthcare Corporation, Pandex Division, 909 Orchard Street, Mundelein, IL 60060.** We are an equal opportunity employer. (NW515)A

Pandex Division

Baxter

香港城市理工學院

City Polytechnic of Hong Kong

TEACHING POSITIONS FROM AUTUMN 1988 IN A RAPIDLY EXPANDING INSTITUTION IN HONG KONG

DEPARTMENT OF APPLIED SCIENCE

Posts & Subject Area:
Principal Lecturer/Senior Lecturer/Lecturer in Physical Sciences/Life Sciences/Environmental Science.

Requirements for Appointment:
A good honours degree or equivalent professional qualification. Also a higher degree and preferably a PhD. Relevant professional, including teaching and research/industrial experience.

Salary Scale:
HK$94,800 p.a. to HK$358,920 p.a. depending on level of appointment. (Exchange Rate: £1 = HK12.86 approx.)

Terms & Conditions:
Excellent fringe benefits are offered including a 25% gratuity payable on successful completion of agreement, medical & dental schemes, and long leave. Passages and housing are provided where appropriate.

Application forms and further information available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Completed forms should reach the General Secretary, City Polytechnic of Hong Kong, PO Box 98441, Tsim Sha Tsui Post Office, Kowloon, Hong Kong by *31 October 1987*: an additional copy should be lodged with the Association of Commonwealth Universities. (W4454)A

Computational Scientist

The Theory and Computational Science Division of the SERC's Daresbury Laboratory has a vacancy for a Computational physicist or chemist to support the Collaborative Computational Project on Heavy Particle Dynamics (CCP6). This project involves a large and active group of university collaborators.

The successful candidate will be expected to collaborate with university groups at Bristol, Durham and Newcastle on the development of theory and computer codes for investigating non-adiabatic processes in atom – atom, ion – atom and ion – molecule collisions.

Applicants should have recently obtained a PhD, or expect to obtain one before taking up the appointment, preferably with experience in quantum chemistry or atomic or molecular scattering theory.

The appointment will be made at a salary between £9,685 and £12,975 according to age, ability and experience. The post will be available for a fixed term of two years and will be superannuable. There is flexibility in the starting date.

Further information can be obtained from:
Dr. C. J. Noble (0925) 603340.

An application form can be obtained from: The Personnel Officer,
Daresbury Laboratory,
Warrington WA4 4AD.
Telephone: 0925 603467
(24 hour answering service).

Please quote reference DL/25.

CLOSING DATE:
Friday 30th October, 1987.

(4162)A

Daresbury
SCIENCE & ENGINEERING RESEARCH COUNCIL

POSTDOCTORAL RESEARCH ASSOCIATE

Protein Biochemistry

At Hoffmann-La Roche, one of the world's largest and most successful researchers and manufacturers of medical and pharmaceutical products, we've spent the last 90 years pushing the boundaries of modern science. In order to maintain this commitment to innovation, we are seeking a Postdoctoral Research Associate to purify proteins and collaborate in structural determinations for proteins which are of pharmaceutical interest (e.g., lymphokine receptors, HIV proteins).

You will investigate factors, such as the elimination of microheterogeneity, which will permit growth of protein crystals that are optimal for structural determinations. This will involve performing final purification steps as well as participating in the planning of genetic engineering and initial stages of protein purification. You will have the opportunity to learn new techniques by collaborating in crystallographic structure determinations and in drug design projects which utilize protein structures.

You should have a PhD in Biochemistry or a related discipline, along with well-developed protein purification skills; experience with FPLC is highly desired.

This position is for one year beginning in early 1988, and may be extended for second and third years. Annual salary is in the $25,000 range. For consideration, please send your curriculum vitae and letter of interest; and have three letters of reference forwarded to: **Dr. Vincent Madison, Leader, Molecular Structure Group, Hoffmann-La Roche Inc., Nutley, New Jersey 07110.** We are an equal opportunity employer.

(NW668)A

ROCHE **Hoffmann-La Roche**

Working Today For A Healthier Tomorrow.

SENIOR FACULTY POSITION IN GENETICS DEPARTMENT OF BIOLOGY THE JOHNS HOPKINS UNIVERSITY

We invite applications or nominations for a tenured position at the full professor level. We are searching for a distinguished individual with an active research program in molecular genetics. The Department of Biology is located in a modern building with outstanding facilities on the Homewood campus of the University.

For further information call 301-338-7285. To apply or nominate, send a curriculum vitae before December 1, 1987 to:

Allen Shearn
Chairman
Genetics Search Committee
Department of Biology
The Johns Hopkins University
Baltimore, Maryland 21218

The Johns Hopkins University is an Equal Opportunity/Affirmative Action Employer.

(NW586)A

EYE RESEARCH INSTITUTE

FACULTY POSITIONS

THE EYE RESEARCH INSTITUTE OF RETINA FOUNDATION

The Eye Research Institute is seeking a new faculty member at the Assistant Scientist level with interests in the mechanisms governing proliferation and migration of retinal pigment epithelium, elaboration of extracellular matrix, or degeneration of the vitreoretinal interface which could be applied to the study of proliferative vitreoretinopathy. Research experience should include cell biology, physiology, or biochemistry. The Institute encourages collaboration with both basic scientists and clinician researchers. Initial funding is available for salary and research costs. Curriculum vitae and letter of interest should be forwarded to:

Sheldon Buzney, MD
Search Committee
Eye Research Institute
20 Staniford St.
Boston, MA 02114

The Eye Research Institute is seeking an additional Assistant Scientist level Ph.D. cell biologist-microbiologist to join active eye research group. Specific areas of interest will involve corneal inflammation, ulceration and wound healing. Experience in cell culture, immunofluorescence and monoclonal antibody techniques required. Salary commensurate with experience. Appointment at Harvard Medical School will be sought. Send curriculum vitae and reprints to:

Kenneth Kenyon, MD
Search Committee
Eye Research Institute
20 Staniford St.
Boston, MA 02114

Equal Opportunity/Affirmative Action Employer
M/F/V/H/and others

(NW576)A

UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE AND MEDICAL BIOTECHNOLOGY CENTER

HUMAN MOLECULAR GENETICIST

The Division of Human Genetics, in conjunction with the University of Maryland Medical Biotechnology Center, is seeking a full-time, tenure-track investigato[illegible] with expertise in mammalian DNA repair at the molecular level. The position requires a PhD degree in Molecular Biology or Mammalian/Human Genetics or with two years of relevant postdoctoral training and experience. Significant research accomplishment and the ability to obtain independent funding are essential. In addition to establishing an independent research program, the successful candidate will be expected to participate in projects involving molecular aspects of the inherited chromosomal instability syndromes. Particular focus will be on the identification, isolation and characterization of genes associated with DNA synthesis and repair in human cells.

The Division of Human Genetics, in both the Departments of Obstetrics and Gynecology and Pediatrics, consists of six full-time faculty members representing all specialty areas and is accredited by the American Board of Medical Genetics. A consortium of additional faculty representing other areas of molecular Biology and Human Genetics research is available at the Medical Center. A training program granting PhD and MS degrees in Human Genetics, as well as postdoctoral research and clinical training, is in place. The successful candidate will have the responsibility of organizing a graduate-level course in Human Molecular Genetics and directing dissertation research of students.

The Medical Biotechnology Center offers a creative and challenging scientific environment, with considerable opportunity for interaction and collaboration with cellular and molecular biologists representing a wide diversity of interests. The Center is a new facility utilizing state-of-the-art techniques and equipment in the pursuit of a broad range of basic and applied scientific projects.

Salary and academic rank are commensurate with qualifications and experience. Applications would include a current curriculum vitae, a detailed statement of past experience and a one page description of future research plans and the names, addresses, and phone numbers of three professional references. Send all applications to: **Maimon M. Cohen, PhD, Chief, Division of Human Genetics Acting Director, Molecular Biotechnology Center, University of Maryland at Baltimore, 655 West Baltimore Street — 11-037 Baltimore, Maryland 21201.**

(NW592)A

POSTDOCTORAL POSITION

available immediately for the molecular study of hepatitis viruses. Experience in Molecular Biology is required. Send CV to:

Dr Basil Rigas,
Division of Digestive Diseases,
Room F-231,
Cornell University Medical Center,
525 East 68th Street,
New York, N.Y. 10021.
(NW566)A

POSTDOCTORAL POSITION

available immediately to participate in the studies on ligand receptors and signal transduction in cultured Leydig cells. Must hold Ph.D. or equivalent degree. Send curriculum vitae and names and addresses of three individuals who can be contacted for reference to: **Dr. Mario Ascoli, The Population Council, 1230 York Avenue, New York, NY 10021.** (NW575)A

Synchrotron Radiation Source:

Head of Scientific Research

The Synchrotron Radiation Source (SRS) at Daresbury Laboratory provides large scale research facilities for the academic and industrial community and supports a vigorous research programme in a wide range of scientific disciplines. The SRS has recently been upgraded and further development is planned. A new post has been created for a senior scientist to manage the experimental research programme.

The Head of Scientific Research will be a senior member of the Laboratory management team responsible to the Director. He/she will manage the overall research programme, develop the experimental facilities and direct the in-house research programme. He/she will maintain contacts with users at home and abroad.

The successful applicant will be an active research scientist.

The Council is prepared to consider a secondment or other type of fixed term appointment.

The salary will be within the scale £24,765 – £28,215. There is a non-contributory superannuation scheme. Some assistance with expenses for house sale and purchase may be available.

Applicants should write to the Director, Professor L. L. Green, no later than 31st October 1987 giving details of qualifications and experience.

Further information about this post is available from the Director at Daresbury Laboratory, Science and Engineering Research Council, Daresbury, Warrington, Cheshire. WA4 4AD.
Telephone 0925-603119.

(4177)A

Daresbury
SCIENCE & ENGINEERING RESEARCH COUNCIL

POSTDOCTORALS, TECHNICIANS, AND GRADUATE STUDENTS IN CARBOHYDRATE CHEMISTRY

The University of Georgia/Department of Energy COMPLEX CARBOHYDRATE RESEARCH CENTER seeks chemists with expertise or interest in learning structural analysis of complex carbohydrates of plants and microbes. Positions available immediately. Salary dependent on training and experience. Send application with complete c.v. to: **Dr Peter Albersheim or Dr Alan Darvill, Co-Directors, UGA/DOE Complex Carbohydrate Research Center, P.O. Box 5677, Russell Lab, Athens, GA 30613, USA.** *AA/EEO institution.* (NW589)A

PROFESSOR SPECIALIZING IN MASS SPECTROMETRY OF COMPLEX CARBOHYDRATES

The University of Georgia/Department of Energy COMPLEX CARBOHYDRATE RESEARCH CENTER has a tenure-track opening for scientist at Associate Professor/Professor level. At least five years' experience in high-mass mass spectrometry required. VG Analytical ZAB SE magnetic sector spectrometer and quadrupole mass spectrometers available. Individual selected expected to develop research team in carbohydrate science. Salary negotiable. Applications received by November 1, 1987 considered at that time. Late applications considered if position not filled. Send letter of application and complete c.v. to: **Dr Peter Albersheim or Dr Alan Darvill, Co-Directors, UGA/DOE Complex Carbohydrate Research Center, P.O. Box 5677, Russell Lab, Athens, GA 30613, USA.** *An EE/EEO institution.* (NW588)E

TENURE TRACK FACULTY POSITION

IN PLANETARY SCIENCES, UNIVERSITY OF COLORADO, BOULDER

The University of Colorado is expanding and broadening its program of research and teaching in planetary sciences. The Department of Geological Sciences and the Laboratory for Atmospheric and Space Physics invite applications for a joint tenure track faculty position in the field of planetary sciences.

We seek a scientist with a record of independent research in one or more of the following areas: 1) Photogeology, morphology, and dynamic process of planetary surfaces; 2) Geochemical evolution of planetary surfaces; 3) Planetary surface-atmosphere interactions. Preference will be given to candidates at the Assistant Professor level but applications from candidates at a higher level will be considered.

The appointment will begin August 1988. Applicants should have a strong commitment to research, to working with graduate students, and to teaching at the graduate and undergraduate levels. To apply, please send a curriculum vita and arrange to have three letters of reference sent to:

Chairman, Planetary Sciences Search Committee, Department of Geological Sciences, Campus Box 250, University of Colorado, Boulder, CO 80309-0250.

Salary: Commensurate with qualifications. Application deadline: 31 October, 1987. Applicants are responsible for making certain that the letters of reference arrive promptly.

The University of Colorado at Boulder has a strong institutional commitment to the principle of diversity in all areas. In that spirit, we are particularly interested in receiving applications from a broad spectrum of people, including women, members of ethnic minorities and disabled. (NW229)A

MINISTRY OF DEFENCE

Clinical Chemist

Up to £21,755

… to take charge of the Clinical Chemistry Laboratory, Royal Army Medical College, London.

Your duties would include management of the laboratory; supervision and teaching of medical laboratory technicians; development of new services for Army hospitals; and research into the medical welfare of the soldier and his family.

A good honours degree or equivalent qualification in an appropriate subject and a wide and up-to-date knowledge of clinical chemistry are essential. A knowledge of quality control and statistical procedures and an appreciation of the role of computers in the modern laboratory are important requirements.

Salary £16,390-£21,755 (including £1465 Inner London Weighting) according to qualifications and experience.

For further details and an application form (to be returned by 16 October 1987) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke (0256) 468551 (answering service operates outside office hours), or telex 859399 CSCOMM G.

Please quote ref: S(H)677.

The Civil Service is an equal opportunity employer

Scientific
CIVIL SERVICE

(4224)A

CHEMIST, ORGANIC

(Research Associate): Require PhD with graduate or industrial experience in use of organoselenium methodologies, amino acids, carbon-ion and photochemical reactions to make heterocyclic natural products and strained carbocyclic compounds. Position involves research in above areas using IR, MS, UV, NMR (^{1}H and ^{13}C) spectrometers, polarimetry and chromatography techniques. $16,000 per year, 40 hours per week. Send résumé, three references from persons familiar with applicant's research to: **Dr A.G. Schultz, Department of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12180.**

Affirmative Action/Equal Opportunity Employer. (NW590)A

NATIONAL INSTITUTES OF HEALTH

Human Monoclonal Antibodies

Staff Fellow Position for M.D., Ph.D., D.D.S., or equivalent to work on human monoclonal antibodies and anti-idiotypic antibodies. Experience in immunology, hybridoma technology and molecular biology is desirable. Candidate should have at least three years postdoctoral experience. Starting salary range, depending on experience, is $24,000 to $46,000 per year. U.S. Citizenship required. The position is available immediately. Send curriculum vitae to **Dr. Lois Salzman, Assistant Scientific Director, National Institute of Dental Research, National Institutes of Health, Building 30, Room 131, 9000 Rockville Pike, Bethesda, Maryland 20892, (301) 496-1483.**

NIH is an Equal Opportunity Employer. (NW392)A

THE UNIVERSITY OF LEEDS

Astbury Department of Biophysics

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post available for a fixed period terminating 31 December 1988 for structural studies on the fungal enzyme galactose oxidase through gene cloning and other methods.

A good honours degree and a PhD in Biochemistry or a related discipline are required.

The project would involve structural studies on the enzyme GALACTOSE OXIDASE isolated from a fungal source. The enzyme has already been purified to homogeneity and crystallized at Leeds. DNA probes to the N-terminal sequence have been made and similar probes to cyanogen bromide fragments of the protein are also being synthesised. It is planned to clone the galactose oxidase gene prior to sequencing and protein engineering studies. The project is in collaboration with Dr M McPherson (Biotechnology), Dr P Stockley (Genetics) and Dr J Findlay (Biochemistry). Attempts might also be made to improve crystallographic conditions for the enzyme so that X-ray crystallographic structural studies can progress. Excellent facilities for all the above aspects of the project are available.

Salary on R/A 1A Grade (£9,305 to £14,825) according to qualifications and relevant experience. Salary scales to be increased wcf 1/3/88.

Informal enquiries may be made to Dr P Knowles (tel: 0532 431751 ext 6536) or Dr M McPherson (tel: 0532 431751 ext 7570).

Application forms and further particulars may be obtained from, and completed applications forwarded to, The Registrar, The University, Leeds LS2 9JT, quoting reference number 83/82. Closing date for applications: 22 October 1987. (4201)A

UNIVERSITY OF BATH

School of Biochemistry

RESEARCH OFFICER

A graduate Research Officer is required to work with Dr G D Holman on the effect of insulin on sugar transport in adipose tissue. The project involves photoaffinity labelling and studying molecular properties of the transport system.

The post is funded by SERC (Membrane Initiative) for three years.

Starting salary up to £8,735 per annum.

Application forms from the Personnel Office, University of Bath, Claverton Down, Bath BA2 7AY or phone Bath 826826 ext. 5033 quoting ref: 87/181. Closing date: 15.10.87 (4199)A

ASTON UNIVERSITY

Department of Pharmaceutical Sciences

Cancer Research Campaign
Experimental Chemotherapy Grou
Pharmaceutical Sciences Institut

INNOVATIVE SYNTHETIC ORGANIC CHEMIST

(REF: 8752)

The Group is seeking an enthu iastic organic chemist to join multi-disciplinary team applyin modern theoretical and exper mental techniques to discove novel antitumour agents. The su cessful applicant will be expecte to contribute to current researc programmes on membrane activ drugs, differentiation-inducin agents and DNA sequence-directe probes but will also be give opportunities to initiate ne research in collaboration with ce biologists and biochemists. A interest in modern biology will b an advantage.

An appointee of suitable exper ience will be given joint respon sibility for the supervision research students and technici and a salary commensurate wit this responsibility will be available.

Commencing salary £9,305 t £14,825 on the Contract Researc Staff Grade 1A scale. The appoint ment is for three years in the firs instance.

Informal enquiries may be dir ected to Professor M F G Stevens Department of Pharmaceutica Sciences, Tel: 021-359 3611, ex 4170.

Application forms and furthe particulars may be obtained from The Personnel Officer (Academi Staff), quoting Ref: 8752/6, Asto University, Aston Triangle, Bir mingham B4 7ET. Tel: 021-35 3611, extension 4563. Closing dat for the receipt of applications 23rd October 1987. (4210)A

UNIVERSITY OF BIRMINGHAM

Department of Physiology

Applications invited for post t work on POMC peptides an phosphatidylinositol metabolism i normal and diseased skeletal muscl Experience in HPLC or lipid bic chemistry an advantage.

Post, funded by Wellcome Tru up to eighteen months. Salary o scale Research Associate 1B c Fellow 1A £8,185 to £11,015 c £9,305 to £14,825 with superann ation. Maximum starting £12,03

Enquiries to Dr M E Smith 472 1301 ext 2446. Applications copies) to: Assistant Registra The Medical School, Birmingha B15 2TJ from whom further pa ticulars are available by 21 Octob 1987. Quote reference RW/Ph MES.

An Equal Opportunities Em lover. (4204)A

Technische Universität Braunschweig

Am *Institut für Geographie* im *Fachbereich für Physik und Geowissenschaften der Naturwissenschaftlichen Fakultät* sind sofort

zwei Professorenstellen

zu besetzen:

Geoökologie und Bodenkunde (Bes.–Gr. C 4)

Bodenphysik und –chemie, Landschaftsinformationssysteme, Fernerkundung, Transport und Umsetzung von Nähr– und Schadstoffen.

Agrarökologie (Bes.–Gr. C 4)

Analyse, mathematische Beschreibung und Simulation komplexer biologischer Kreisläufe.

Die zu Berufenden sollen an der Ausgestaltung des Studienganges Geoökologie mitwirken und sich an leitender Stelle im Sonderforschungsbereich "Wasserund Stoffdynamik in Agrar-Ökosystemen", dessen Sprecher der verstorbene Professor Heinrich Rohdenburg war, beteiligen. Beide sollten systemanalytisch mit dynamischen Modellen arbeiten können. Als Bewerber kommen außer Geographen auch Vertreter angrenzender Fachgebiete (z.B. Bodenkunde, Agrarwissenschaft, Biologie) in Frage. Von den Stelleninhaber(n)/innen wird eine Beteiligung an der Ausbildung von Dipl.–Geographen erwartet.

Die allgemeinen Einstellungsvoraussetzungen sind in § 56 des Niedersächsischen Hochschulgesetzes geregelt, dessen Text auf Anforderung zugesandt wird. Schwerbehinderte werden bei gleicher Eignung bevorzugt.

Qualifizierte Bewerber werden gebeten, ihre Bewerbung (mit Lebenslauf, Schriftenverzeichnis und Angaben über die bisherige Lehr–und Forschungstätigkeit) bis 6 Wochen nach Erscheinen dieser Anzeige einzureichen beim *Vorsitzenden der Naturwissenschaftlichen Fakultät der Technischen Universität Braunschweig, Prof. Dr. Herbert Dreeskamp, Pockelsstraße 14, D-33oo Braunschweig.* (W4463)A

BIOTECHNOLOGIST

Immunoscope is a Research and Development Company involved in immunodiagnostics. The Company is investing in a new laboratory facility in Leeds to specifically work with the development and use of novel immunoassays.

We are now seeking a Biochemist with experience in immunology. It is anticipated that the successful candidate will play a leading role within the company's research programme. Initially you will be working independantly within a small team.

The company offers an attractive salary by negotiation together with a number of fringe benefits.

Applications should be made in writing together with a curriculum vitae and present salary to:

Mr Michael Lofthouse, General Manager, Immunoscope Limited, Deacon House, Seacroft Avenue, Leeds, West Yorkshire LS14 6JD.

(4214)A

ACADEMIC FACULTY POSITION IN BIOCHEMISTRY AND MOLECULAR BIOLOGY

MAYO CLINIC, MAYO MEDICAL SCHOOL AND MAYO GRADUATE SCHOOL OF MEDICINE

Department of Biochemistry and Molecular Biology seeks applications for a faculty appointment at the assistant, associate, or full professor level, depending on qualifications. Preference will be given to outstanding molecular or developmental biologists with demonstrated expertise in somatic cell and germ line gene transfer techniques. Highly qualified individuals with other molecularly oriented research interests are also encouraged to apply. The Mayo Clinic offers an outstanding research environment in a medical and academic setting. Send curriculum vitae, statement of research interests, and names of three references by February 15, 1988 to **Dr Michael J. Getz, Chairman, Search Committee, Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, MN 55905.**

An equal opportunity/affirmative action institution. (NW577)A

CSIRO

INDUSTRIAL STATISTICIAN

A$28,629–A$41,861

DIVISION OF MATHEMATICS & STATISTICS MELBOURNE VIC

THE DIVISION: The Division of Mathematics and Statistics has a staff of approximately 80 including 61 professional scientists. The Division's headquarters are at Canberra, and there are Regional Offices in Melbourne, Sydney, Adelaide, Brisbane and Perth. Scientists of the Division consult on the statistical, mathematical and numerical aspects of research projects undertaken by scientists in other CSIRO Divisions, Government Departments and Industry; engage in collaborative research in science and technology with CSIRO and Industry; and conduct related research in statistics and mathematics.

THE JOB: The appointee will be attached to the Division's Industrial Statistics Project with a major objective of fostering excellent statistical practice in Australian Industry, especially in the area of quality improvement. He/she will be expected to take responsibility for making direct contacts with Industry in Melbourne, and to consult and collaborate, both directly and through SIROMATH, with industrial groups, individual companies and relevant organisations with a view to integrating statistical thinking into quality improvement programs. (The position may also entail collaboration with other CSIRO Divisions, especially the Division of Manufacturing Technology.) The appointee will also be expected to contribute to the Project's research into aspects of statistical methodology relevant to quality improvement.

THE PERSON: A PhD degree or equivalent qualifications in a branch of statistics and a proven record of independent statistical consulting and an extensive research record. Preference will be given to applicants with experience in consulting directly with Industry, possessing a high level of drive and initiative and familiarity with the application of statistical ideas in total quality management. Consideration may be given to the appointment of an outstanding applicant at a higher level.

CONDITIONS: Appointment will be for a term of three years with Australian Government Superannuation benefits available.

LOCATION: In Melbourne, the Division of Mathematics and Statistics is located at Clayton, but the position requires substantial travel inthe Melbourne area.

MORE INFORMATION: Prospective applicants are invited to telephone Dr. Ron Sandland (02) 467 6063 for further information. A copy of the job description and selection criteria can be obtained from Dr Sandland or at the address below.

APPLICATIONS: Applications should be submitted by 16th December 1987 and should quote reference number A1580. They should take into account the selection criteria, and should state relevant personal particulars, including details of qualifications and experience. Applicants should nominate at least two professional referees and address their applications to:

The Chief
CSIRO Division of Mathematics & Statistics
GPO Box 1965
Canberra ACT 2601
Australia

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W4467)A

FACULTY POSITION IN MICROBIOLOGY ASSISTANT PROFESSOR, TENURE-TRACK

Applicants should have postdoctoral experience and demonstrated research capability. The successful applicant will be expected to establish an independent, externally-funded research program focusing on a significant problem in microbiology, with a preference for the basic biology of microbial pathogenesis using physiological, molecular, or genetic approaches. Teaching responsibility will include an undergraduate/graduate level course in human pathogenic microbiology. Position available August, 1988. Send curriculum vitae, statement of research interests, and three letters of recommendation to: **Dr. Dwane Wylie, Microbiology Search Committe, School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 68588-0118.** Applications will be reviewed November 9, but will be accepted until a suitable candidate is found.

Affirmative Action/Equal Opportunity Employer

(NW565)A

CSIRO

STATISTICIANS

(2 positions)

A$28,629–A$41,861

DIVISION OF MATHEMATICS & STATISTICS
MELBOURNE VIC

THE DIVISION: The Division of Mathematics and Statistics has a staff of approximately 80 including 61 professional scientists. The Division's Headquarters are at Canberra, and there are Regional Offices located at Melbourne, Sydney, Adelaide, Brisbane and Perth. Scientists of the Division consult on the statistical, mathematical and numerical aspects of research projects undertaken by scientists in other CSIRO Divisions, Government Departments and Industry; engage in collaborative research in science and technology with CSIRO and Industry; and conduct related research in statistics and mathematics.

THE JOB: The appointees, as members of the Melbourne region of the Division, will undertake statistical research appropriate to their experience and expertise, and will consult and collaborate in research with scientists in other CSIRO Divisions in the region and industry.

THE PERSON: A PhD degree or equivalent qualifications in a branch of statistics and an extensive research record. Preference will be given to applicants with consulting experience in science or technology and/or experience collaborating with industry. Appointment at a higher salary level than the above range may be considered for an outstanding applicant.

CONDITIONS: One position (A3556) is available on an indefinite basis. In addition, one research fellow position (A3578) is available for a fixed term of three years. Australian Government Superannuation benefits are available for both positions.

MORE INFORMATION: Prospective applicants are invited to telephone Miss Angela Johnstone on (062) 81 8536 for further information. A copy of the job description and selection criteria can be obtained from Mrs M Hepworth on (062) 81 8534 or at the address below.

APPLICATIONS: Applications should be submitted by 30th October 1987 and quote the appropriate reference No(s). They should take into account the selection criteria, and should state relevant personal particulars, including details of qualifications and experience. Applicants should nominate at least two professional referees and address their applications to:

The Chief
CSIRO Division of Mathematics & Statistics
GPO Box 1965
CANBERRA CITY ACT 2601
AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W4466)A

ETH ZÜRICH

POSTDOCTORAL RESEARCH FELLOW

Postdoctoral position immediately available. Project involves studies on the use of cellular receptors and transport systems for the uptake of drugs and drug carriers, i.e. liposomes. Recent PhD with training in biophysics, biochemistry or cellular biology preferred. Send curriculum vitae and three letters of reference to:

Dr Heidi Wunderli-Allenspach, Assistant Professor, Department of Biopharmacy, Pharmaceutical Institute, Federal Institute of Technology, CH-8092 Zürich, Switzerland.

(W4460)A

UNIVERSITY OF HONG KONG

(Re-advertisement)

CLINICAL PATHOLOGIST (BIOCHEMISTRY)

Applications are invited for a newly established post of Clinical Pathologist in the Clinical Biochemistry Unit, Hospital Pathology Services of the University. Applicants should possess a recognised medical degree with appropriate postgraduate training and experience in chemical pathology and a background of research in related problems. Applicants studying for the MRC Path or equivalent will be considered but preference will be given to those who have attained a higher qualification. The appointee will work in the Clinical Biochemistry Unit which provides chemical pathology service the Queen Mary Hospital under the supervision of a Senior Consultant.

Annual salary (superannuable) is on a 13-point scale: HK$173,640 to $358,920 (approx £13,570 to £28,040; sterling equivalent as at September 15, 1987). Starting salary will depend on qualifications and experience. At current rates, salaries tax will not exceed 16½% of gross income. Housing benefits at a rental of 7½% of salary, children's education allowances, leave and medical benefits are provided.

Further particulars and application forms may be obtained from: The Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from: The Appointments Unit, Registry, University of Hong Kong, Hong Kong. Closes 15 November 1987. (W4462)A

SANDWELL HEALTH AUTHORITY

Department of Neurosurgery,
University of Birmingham

RESEARCH TECHNICIAN

(£8,346 to £11,026 pa)

To participate in a three year programme funded by the West Midlands Regional Health Authority. The project is in the field of neuronal and glial cell growth. The candidate should have a degree of biological science or appropriate basic discipline and experience in cell culture and/or immunological techniques. Experience in immunohistochemistry an advantage.

For further details and application form please contact Professor Hitchcock or Veronica Turner at the Midland Centre for Neurosurgery and Neurology, Holly Lane, Smethwick, Warley, West Midlands B67 7JX, England. Telephone 021-558 3232. Applications should be accompanied by CV and names and addresses of two referees. Closing date for applications 9th October 1987.

Sandwell is a non-smoking health authority. (4203)A

UNIVERSITY OF BIRMINGHAM

Department of Cardiovascular Medicine

The newly established Britis Heart Foundation Molecula Cardiology Research Group ha vacancies for a

RESEARCH ASSOCIATE & RESEARCH TECHNICIA

The appointments funded by th Wellcome Trust are to develo radionuclide imaging technique for diagnosis of cardiovascula disease using labelled monoclona antibodies. Other techniques inc lude cell culture, HPLC, surgery cell sorting (FACS), ELISA. Th research has commercial produc implications.

Preferred experience in bio chemical or immunochemica techniques and cell culture tech niques for Technician post.

Qualifications: Research Assoc iate, degree in biochemistry, biological sciences or related subjec and 1-2 years' practical experience Research Technician post, mi mum ONC in relevant subject.

Research Associate post available for 2 years from 1 October or 1B scale (£8,185 to £11,015) maximum starting salary of £8,735. Research Technician salary range (£6,314 to £7,448).

Informal enquiries from the Unit Director, Dr P Cummins, telephone 021-472 1301 extension 2401.

Applications (3 copies) including curriculum vitae and names of two referees should be sent to Assistant Registrar, Medical School, University of Birmingham, Birmingham B15 2TJ as soon as possible. Quote reference RP/CVM/PC.

(4194)A

UNIVERSITY OF LONDON

St Georges Hospital
Medical School

TECHNICIAN GRADE III

Department of Anatomy

Two posts to work with Dr Alu Davies on neuronal developmen in vertebrate embryos are availabl for 2 and 3 years, respectively. On post involves mainly recombinan DNA and biochemical technique and the other involves mainl microdissection and tissue culture Preference will be given to graduates in biochemistry, physiology zoology and related subjects. Th opportunity to read for a PhD i available for motivated and suitab qualified persons. Starting salar £6,314 plus £840 London All ance.

Application forms and furthe details are available from: Th Personnel Officer, St George Hospital, Cranmer Terrac Tooting, London SW17 0RE Closing date: 22 October 198 Quote reference 129/87.

(4208)A

UNIVERSITY OF LONDON

St Georges Hospital
Medical School

RESEARCH FELLOW

Department of Clinical
Epidemiology & Social Medicine

Applications are invited for the post of research fellow for an MRC funded survey of rhinitis (hayfever) in the community. This is a full time post with duties to include the day-to-day administration of the project, training and supervision of interviewers, and data preparation and analysis. The appointment is for 18 months. Work will be carried out under the direction of the principal investigator.

Applicants should have a post-[illegible]raduate qualification and approp-[illegible]ate research experience in medical [illegible] related fields. Starting salary [illegible] range, according to age and [illegible]erience, from £9,865 to £11,015 [illegible] £1,393 London Allowance on [illegible] research fellow grade 1A/B [illegible]e.

[illegible]pplication forms and further [illegible]articulars are available from: The [illegible]ersonnel Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. Closing date: 22 October 1987. Please quote reference 130/87.

(4207)A

PHARMACOLOGIST

Cerebrovascular Disease

Pfizer, one of the world's foremost and highly respected research based pharmaceutical companies, has an attractive opportunity for a young post-doctoral pharmacologist to join and influence a new research project from the start.

In this important new position you will be able to make a significant contribution to the development of ideas and methodology required in the discovery of novel therapies for cerebrovascular disease.

If you have about 3 years relevant post doctoral experience, ideally involving the study of ion transport systems in cell membranes, this is an ideal chance to further develop your career in a challenging and intellectually stimulating environment.

In addition to a strong scientific background you should have the interpersonal skills to ensure effective interactions with other scientists in a multi-disciplinary project team.

Pfizer

We are offering an excellent remuneration and benefit package including bonus related to personal performance and generous assistance in relocating to an attractive area of coastal Kent.

If you enjoy the challenges of discovery research and would like to take this career opportunity please write in confidence, quoting reference CPN, giving full details of career to date to Mr D O'Callaghan, Personnel Department, Pfizer Central Research, Ramsgate Road, Sandwich, Kent CT13 9NJ.

(4230)A

GEOLOGY:

Full-time tenure-track position in geomorphology and glacial geology beginning August 1988. PhD and strong quantitative orientation required. Duties involve teaching undergraduate courses and conducting research. Send curriculum vitae and letters of recommendation by 15 December 1987 to: **Professor George H. Springer, Geology Department, University of Dayton, Dayton, OH 45469. Telephone: 513-229-2921.**

Affirmative Action/Equal Opportunity Employer.

(NW571)A

NEUROBIOLOGY IN PUERTO RICO

Faculty (tenure track, rank open) and Postdoctoral positions are available for individuals with research interests in membrane, cellular and systems neurobiology (biophysics, electrophysiology and histology). Academic appointments will be in a relevant Medical Sciences Campus department. Limited teaching in either Spanish or English. Letters of application with a c.v., reprints and the names of three referees should be sent to **Dr Richard K. Orkand, Institute of Neurobiology, University of Puerto Rico MSC, 201 Blvd. del Valle, Old San Juan, Puerto Rico 00901, USA.**

For information in the UK please direct enquiries to Professor David S. Smith, Zoology Department, Cambridge University, Cambridge, UK. *EOAAE.*

(NW585)A

ATMOSPHERIC CHEMIST/PHYSICIST

UNIVERSITY OF CALIFORNIA AT LOS ANGELES (UCLA). The Department of Atmospheric Sciences invites applications for a junior or senior level (assistant, associate, or full professor) ladder faculty appointment. Preference will be given to applicants with a proven record of research accomplishments in the areas of atmospheric chemistry, radiation and cloud physics. The position entails developing a strong research program with an emphasis on graduate guidance. The department also considers superior performance in graduate and undergraduate teaching to be important.

Interested persons should submit their curriculum vitae, publication list and three references before November 1, 1987 to:

George L Siscoe
Chairman
Department of Atmospheric Sciences
UCLA
Los Angeles, CA 90024

UCLA is an Affirmative Action/Equal Opportunity Employer.

(NW358)A

RESEARCH SCIENTIST — DNA/PROTEIN CHEMIST

The University of Arizona has an opening for a RESEARCH SCIENTIST with experience in DNA and/or PROTEIN CHEMISTRY. Applicants should have a PhD or equivalent degree in some branch of chemistry or biological sciences. Duties will involve managing a core facility, equipped with state-of-the-art automated sequence and synthesis instruments, and conducting independent research. The facility has a technical support staff and operations financed by the University. The successful applicant will be expected to apply for grants to support his/her research program. The position is continuing or continuing eligible, depending upon qualifications. Salary is open, subject to negotiation. Applications will be examined after November 30, 1987, but will be accepted until the position is filled. Address applications to **Dr John H. Law, Biotechnology Program, Biosciences West, University of Arizona, Tucson, AZ85721.** *The University of Arizona is an Equal Opportunity/Affirmative Action Employer.*

(NW594)A

Assistant Research Physicist or Astronomer

EUV Instrumentation and Physics

The Space Astrophysics Group at the University of California, Berkeley has two positions available for Research Scientists. The positions are for 2 years beginning November 1987 with a high likelihood of extension. One position is for the development of thin film filters and the second is to supervise the calibration, testing and development of instruments for Extreme Ultraviolet Explorer Satellite. The positions will require theoretical and analytic work in the design and testing of novel instrumentation for use in the EUV.

A Ph.D. in Physics or Astronomy or related fields with experience in space instrumentation is required. Laboratory experience with X-Ray and EUV gratings, spectrometers, detectors and other testing and calibration techniques is helpful.

Send résumé and three letters of recommendation by October 31, 1987 to:

Dr. Roger Malina
Space Sciences Laboratory
University of California
Berkeley, CA 94720

The University of California is an Equal Opportunity, Affirmative Action Employer.

(NW555)A

TWO POSTDOCTORAL RESEARCH ASSOCIATES

Two positions are available to study the biochemistry, regulation and pathology of red blood cell membranes and membrane-skeletal proteins. Applicants with experience in protein biochemistry, biological membranes, protein kinases and phosphates are encouraged to apply. Opportunity to join an established group in a well equipped and active department. One position will be available immediately and the other in January, 1988. A highly competitive salary appropriate to experience will be offered. Send curriculum vitae and names of three references to: **Carl M. Cohen Ph.D., Chief, Division of Cellular and Molecular Biology, St. Elizabeth's Hospital, 736 Cambridge Street, Boston, MA 02135.** EOE. (NW580)A

TWO POSTDOCTORAL FELLOW/ RESEARCH ASSOCIATE POSITIONS AVAILABLE IMMEDIATELY

One position is for work on the role of cyclic nucleotides and calcium in the control of intestinal electrolyte transport. The second position is for work on the regulation of insulin release in pancreatic B-cells. Candidates should have Ph.D. or equivalent degree with experience in biochemistry, physiology or pharmacology. Applications including curriculum vitae and the names of three references should be sent to:

Dr Geoffrey W.G. Sharp, Chairman
Department of Pharmacology
NYS College of Veterinary Medicine
Cornell University
Ithaca, NY 14853-6401

Applications must be received by November 15, 1987
Salary Range: $17,600-£19,500

Cornell University is an Affirmative Action/Equal Opportunity Employer/Educator (NW569)A

UNIVERSITY OF SURREY

Department of Microbiology

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a postdoctoral research fellow to work on the development of improved procedures for the production and assay of the unusual peptide antibiotic nisin, which is used as a food preservative. This project will involve a wide range of techniques including monoclonal antibody production, peptide chemistry and gene closing. Experience in one or more of these fields would be an advantage, but the prime requirement is for the ability to face up to a challenge and to acquire new skills. Candidates without a PhD but with suitable experience/abilities may also be considered.

The project is funded by the AFRC and the appointment will be for 3 years. Salary will be on the Research & Analogous 1A scale at a starting point between £9,305 and £11,015 depending on age, qualifications and experience. Superannuation under USS conditions.

Further details can be obtained from Dr M R Adams (0483) 571281 ext 2669 or Dr J W Dale ext 2653.

Applications in the form of a curriculum vitae (3 copies) including the names and addresses of two referees should be sent to: (JLG) Personnel Office, University of Surrey, Guildford, Surrey GU2 5XH by 22 October 1987, quoting reference no 657/N. (4205)A

UNIVERSITY OF LONDON

St George's Hospital Medical School

RESEARCH TECHNICIAN – DEPARTMENT OF HAEMATOLOGY

Applications are invited for the post of Research Technician to work with Dr Rutherford on a project to study the molecular biology of human globin gene regulation. The project will involve tissue culture, recombinant DNA and gene transfer techniques. Experience in these areas is desirable but not essential as training will be given.

Candidates should have an honours degree in an appropriate biological science, or substantial relevant experience.

The post is funded by the Wellcome Trust for a period of three years.

Salary range: (£6,314 to £8,346 plus £1,393 London Allowance) depending on age and experience.

Further particulars and an application form from the Personnel Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. 01-672 9944 ext 56020. Closing date: 22nd October, 1987. Quote reference 125/87. (4182)A

UNIVERSITY OF WARWICK

LECTURESHIP IN DEVELOPMENTAL BIOLOGY

Applications are invited for a Lectureship in Developmental Biology in the Department of Biological Sciences, from persons with good qualifications and an interest in studying animal development using molecular and/or genetic techniques. Particular preference will be given to candidates applying molecular approaches to genetically well-characterised systems, such as Drosophila. In addition to assisting in undergraduate teaching, the successful candidate will be expected to develop a programme of research which complements an existing group o[illegible] developmental biologists, in th[illegible] Animal Molecular Genetics R[illegible] search Group, working on [illegible] genetic control of animal devel[illegible] ment. Excellent facilities fo[illegible] search in this area already [illegible] Further information may b[illegible] tained from Professor H R W[illegible] land (Tel 0203 523536).

Salary will be up to £12,6[illegible] on the Lecturer A Scale £8,73[illegible] £13,675 pa (plus approximately a further 6% from 1st March 1988) according to age, qualifications and experience, with the expectation of progression to Lecturer B (Max £18,210).

Further particulars and application forms from the Registrar, University of Warwick, Coventry CV4 7AL (0203 523637) quoting Ref No 7/A/87/O (mark clearly on envelope). (4174)A

ROYAL COLLEGE OF SURGEONS OF ENGLAND

Hunterian Institute

Department of Pharmacology

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the position of Postdoctoral Research Assistant in the Department of Pharmacology. The post is funded by the Medical Resarch Council for 3 years and the successful candidate will join a team studying inflammation and hyperreactivity of the airways.

Candidates should possess or be completing a PhD in Pharmacology, Physiology or Biochemistry, although those with equivalent experience will also be considered. Initial salary range £9,305 to £10,440 pa plus £1,393 London Allowance.

Applications, including a f[illegible] curriculum vitae and the names of three referees should be sent to Assistant Secretary (Personnel) Royal College of Surgeons of England, 35-43 Lincoln's Inn Fields, London WC2A 3PN.

Please quote ref. 45/87. Closing date: 22nd October 1987.

(4187)A

MRC

Medical Research Council

Anatomical Neuropharmacology Unit

Immunochemist/Immunocytochemist

Applications are invited for a SHORT TERM NON-CLINICAL SCIENTIFIC post in this MRC Unit, tenable for 3 years. Candidates of immediately postdoctoral or equivalent status will be preferred (but well qualified candidates with less than 3 years postgraduate experience will also be considered).

The successful applicant will have experience in the production and use of monoclonal antibodies to identify cell type specific markers for use in the biochemical and morphological characterization of functionally distinct neuronal populations. Knowledge of the structure of the central nervous system and experience in the preparation of antibody conjugates would be advantageous.

Remuneration will be at an appropriate point on the scales for university non-clinical academic staff. Further information and an application form may be obtained from **Dr. R.A.J. McIlhinney, MRC Anatomical Neuropharmacology Unit, Dept. Pharmacology, South Parks Road, Oxford, OX1 3QT**, with whom applications — including a full CV and the names of two professional referees — should be lodged by 1/12/87. (4184)A

HUMAN MOLECULAR GENETICIST

The Division of Medical Genetics, Emory University School of Medicine, is seeking applications for a tenure-track assistant professor position. The successful candidate is expected to participate in ongoing genetic linkage studies using RFLPs, to develop an independent research program in some aspect of human postdoctoral training. To apply, send a curriculum vitae and a synopsis of current and future research interests to:

Dr. Louis J. Elsas, II, Director
Division of Medical Genetics
Emory University
2040 Ridgewood Drive, Room 130
Atlanta, GA 30322

Deadline for receipt of applications is December 1, 1987. (NW550)A

MACROMOLECULAR CRYSTALLOGRAPHY

University of California, Berkeley

The Department of Chemistry and the Department of Biophysics invite applications for two faculty positions (pending final budgetary approval), one at the Associate Professor or Professor level and the other at the Assistant Professor level. The appointments are to be effective within the next two years.

Applicants should have a PhD degree or equivalent and relevant prior experience in X-ray crystallography of biological macromolecules. Faculty duties will include research in structural molecular biology, supervision of graduate student and postdoctoral research, and teaching at the undergraduate or graduate levels. The successful candidate will be part of an expanding, interdisciplinary structural biology group at Berkeley that also includes members of the departments of Molecular Biology and Biochemistry.

Applications should include: a letter specifying qualifications and current research interests, curriculum vitae, a list of publications, representative reprints, and the names and addresses of persons whom we may contact for letters of recommendation.

Applications will be processed as soon as they are received but should arrive no later than February 15, 1988. They should be addressed to: Professor Robert Glaeser, Biophysics Department, Donner Laboratory, University of California, Berkeley, CA 94720.

The University of California is an Equal Opportunity Affirmative Action employer. (NW602)A

ROCHE

To strengthen the Pharmacokinetic Group of our Clinical Research Department we are seeking a

PHARMACIST (PhD, PharmD)

Or

a Physician (MD)

who has an interest in biopharmaceutical research and had prior exposure to pharmacokinetics.

The group is responsible for the investigation of the pharmacokinetic profile of new drugs in animals and humans from the early development phases up to their introduction on worldwide markets. The work includes planning, conducting and evaluating pharmacokinetic studies and requires a good command of written and spoken English as well as the willingness to learn German.

Interested applicants may direct their complete application documentation including a handwritten c.v. to our Personnel Department, quoting reference Nature 203/87/WA.

F. Hoffmann-La Roche & Co. Limited Company,
CH-4002 Basle (W4459)A

The Cancer Research Program at the City of Hope National Medical Center is seeking a recent PhD with an interest in the study of human solid tumors for a tenure-track position at the level of

ASSISTANT RESEARCH SCIENTIST

Preference will be given to people with research interests and experience in gene plasticity, structure, or expression. The City of Hope offers a competitive salary (commensurate with qualifications) and an excellent fringe benefit package. The Cancer Program offers access to excellent and modern facilities for DNA synthesis, protein sequencing, molecular modeling, and molecular cloning. Collaborative research is encouraged. Send curriculum vitae, and a brief summary of research objectives to:

DR. STEVEN S. SMITH,
Division of Surgery,
City of Hope National Medical Center,
1500 East Duarte Road, Duarte, CA 91010.

Affirmative Action/Equal Opportunity Employer. (NW607)A

Scripps Institution of Oceanography: Mantle Geochemistry of Rare Gases and Stable Isotopes

Applications are invited for a Post-doctoral Research Scientist to work on studies of volatiles (rare gases, C, H, N) in mantle rocks, diamonds, volcanic gases, etc. Applicants should have a PhD in physics, nuclear geophysics, or nuclear geochemistry. Experience with rare gas mass spectrometry and vacuum systems is a plus, as is any exposure to terrestrial hard rocks. Send CV and ask for letters to be sent, to **H. Craig/D. Lal, Geological Research Division A-020, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093.**

Appointment at level depending on experience is possible January 1, 1988.

Equal Opportunity, Affirmative Action.

Exciting work, and not a bad place to live. (NW600)A

UNIVERSITY OF KENT AT CANTERBURY ■■■■

Chemical Laboratory

Research Fellow – Mass Spectrometry

Applications are invited for a post-doctoral Research Fellowship to undertake a two year investigation into aspects of environmental atmospheric analysis employing direct sample introduction into a Finnigan ion trap mass spectrometer (ITD).

For this project, which is funded by the Ministry of Defence, applicants should ideally have practical 'hands-on' experience in gas chromatography and/or mass spectrometry, with a bias towards instrumental development. Salary in the range £9,305-£11,460 (1A Scale).

Further details are available from Dr J F J Todd, Chemical Laboratory, University of Kent, Canterbury (Tel: 0227-764000 Ext. 3518).

Apply by letter with a full *curriculum vitae,* including the names of two academic referees to **Mr I R Stone, Senior Assistant Registrar, Faculty of Natural Sciences, The Registry, University of Kent, Canterbury, Kent CT2 7NZ quoting reference A88/9.** Closing date 24 October 1987. (4213)A

Postdoctoral and Postgraduate Research Positions in Plant or Fungal Cell Biology

The successful candidates will join a multidisciplinary research program devoted to plant-fungal interactions and fungal (yeast) and plant cell biology.

Qualifications: Postdoctoral positions, a Ph.D. in plant or fungal biochemistry/physiology or in a related field, preferably with experience in enzymology and protein chemistry. Postgraduate positions, a M.Sc. or equivalent degree in botany or microbiology.

Conditions: Postdoctoral positions, salary in the range of SFr. 45,000-60,000 p.a. (depending on experience) for up to five years. Postgraduate positions, salary SFr. 20,400 p.a. for three years; research is expected to culminate in submission of a thesis as required for the title of Doctor of Natural Sciences. Postdoctoral and postgraduate research assistants will be expected to participate in graduate and undergraduate teaching.

Send c.v., names of two references, relevant reprints to:

Prof. Dr. Th. Boller & Prof Dr. A. Wiemken
Abteilung Pflanzenphysiologie
Universität Basel
Hebelstrasse 1
CH-4056 Basel, Switzerland

(W4465)A

UNIVERSITY OF OXFORD

Nuffield Laboratory of Ophthalmology

Postdoctoral Research Assistant — Grade 1A

Applications are invited for the above position to work on a project concerning the physicochemical properties of Meibomian lipids and their role in promoting stability of the preocular tear film, under the direction of Dr. J.M. Tiffany. The post is supported by the Medical Research Council for three years on a salary scale from £10,440 to £11,460.

The project will involve some lipid synthesis and collection of natural lipid secretions, and their use in physical measurements of properties such as melting-point, viscosity and evaporation-control which depend on composition and which help to determine the function and effectiveness of the Meibomian secretion in the eye. The work will require a broad range of biochemical, physical and biological techniques; previous experience in lipid chemistry or physical chemistry would be an advantage, but is not essential.

Applications, which should include a curriculum vitae and the names of two referees, should be sent as soon as possible to **Dr. J.M. Tiffany, Nuffield Laboratory of Ophthalmology, Walton Street, Oxford OX2 6AW, UK.,** from whom further details may be obtained. (4212)A

AFRC INSTITUTE OF ARABLE CROPS RESEARCH

Rothamsted Experimental Station
Harpenden Herts AL5 2JQ

MOLECULAR GENETICIST – BARLEY GENOME MAPPING

Applications are invited for a Higher Scientific Officer to join a team in the Biochemistry Department working on the mapping of the barley genome using DNA RFLP analysis. The person appointed will be responsible for the organisation of the programme. Technical support will be provided by an ASO. Previous experience of Molecular Biology or Molecular Genetics is essential.

The project is supported by Shell Research Ltd, in conjunction with Nickerson RPB Ltd, and the post is available immediately for up to three years.

Qualifications: First or Upper Second Class Degree in Biochemistry or a related subject with at least two years post-qualifying experience. PhD preferred.

Salary on scale £9,219 to £12,505. Non-contributory superannuation.

Applications, giving full career details and the names of two referees and quoting Ref 694 should be sent to The Secretary by 16 October. Further details on request. Informal telephone enquiries can be made to Dr P R Shewry (05827 63133).

We are an equal opportunities employer. (4179)A

THE INSTITUTE OF HORTICULTURAL RESEARCH

Littlehampton

TEMPORARY SCIENTIFIC OFFICER

(HDC Funded)

A Scientific Officer post is available to work on 'Tomato Fruit Quality — pre- and post-harvest Factors'. Duties include laboratory and glasshouse work in connection with growing tomatoes in recirculating solution culture, both at Littlehampton and at Experimental Horticulture Stations.

Qualifications: Degree, HNC or equivalent with relevant experience.

Salary in scale: £7,816—£10,154.

Further details from the Personnel Officer, Institute of Horticultural Research, Bradbourne House, East Malling, Kent ME19 6BJ, quoting ref: 13/L by 12th October 1987.

The Institute of Horticultural Research is an Equal Opportunities Employer. (4167)A

UNIVERSITY OF YORK

Department of Physics

POSTDOCTORAL RESEARCH ASSISTANT I THIN FILM PHYSICS

Applications are invited for
EEC-funded post, which is ava
able for a period of three ye
beginning anytime after 1 Novem
1987, to work on the preparati
structure analysis and dynan
magnetic properties of metal
ferromagnetic multilayers. T
work is in collaboration wi
CNRS, Bellevue, France and t
University of Paris VII, with whi
some interchange is envisage
The experimental techniques to
employed will include ultra hi
vacuum evaporation, RHEE
Auger spectroscopy, microwa
spectroscopy and magnetomet
Experience in ferro-magnetism
UHV techniques/systems and/
of computer control of experime
would be an advantage.

Salary within the range £9,3
to £12,305 (or one point higher
exceptionally well qualified app
cants) per annum, with USS.

Three copies of application
with full curriculum vitae a
naming two referees, should
sent by 30 October 1987 to Reg
trar's Department (Appointment
University of York, Heslingto
York YO1 5DD. Further par
culars are available. Please quo
reference number 7/6242.

Informal enquiries to Dr
Chambers (0904 430000 e
5536). (4217)A

UNIVERSITY OF LEICESTER

DEPARTMENT OF GENETICS

UNIVERSITY OF BATH

MICROBIOLOGY GROUP

RESEARCH ASSISTANT

Graduate in Biological Scien
required to work on a SER
funded project to study plasn
maintenance in yeasts using no
techniques for the analysis of in
vidual cells. The project is part
collaboration between Dr A
Wheals at Bath and Dr Anne
Cashmore at Leicester. The s
cessful candidate will spend ti
working at both institutions,
will be based at Leicester. If s
able, he/she may register fo
higher degree.

The position is for 3 years co
mencing 1st January, 1988. Sal
on the 1B range for research
analogous staff (£8,185—£11
p.a.)

Applications with curricu
vitae and names of two refe
should be sent as soon as poss
to Dr A.M. Cashmore, Dep
ment of Genetics, Universit
Leicester, Leicester LE1 7
telephone no. 0533 523439.
(4168)

UNIVERSITY OF ALBERTA
DEPARTMENT OF PHARMACOLOGY

The University of Alberta, Department of Pharmacology, is seeking applications for a tenure-track faculty position at the level of assistant professor. (Salary range $31,612—$45,340). Applicants should have a PhD in pharmacology or a related discipline, and be highly qualified investigators capable of directing a strong program of independent research. They must be enthusiastic and competent teachers. The University of Alberta is committed to the principle of equity in employment. This position will be available immediately. Closing date for applications is November 30, 1987.

Those interested should apply in writing enclosing a curriculum vitae, the names of at least three referees, and any other relevant information to:

Dr D.A. Cook, Chairman
Department of Pharmacology
9-70 Medical Sciences Building
Faculty of Medicine
University of Alberta
Edmonton, Alberta T6G 2H7
Telephone (403) 432-3575 (NW573)A

POSTDOCTORAL RESEARCH POSITION:
MEMBRANE PROTEIN SYNTHESIS

A research position is available for a PhD or MD candidate to join the team which is actively working on mammalian skeleton protein synthesis and its defects in hereditary anemias. Candidates should have a background in protein chemistry and in cell culture techniques, including immunochemistry with emphasis on monoclonal antibodies.

POSTDOCTORAL RESEARCH POSITION:
ELECTRON MICROSCOPY

Available immediately to candidates with skill in cell biology/electron microscopy to study ultrastructural aspects of red cell skeletal protein biogenesis and molecular defects of membrane skeletons in abnormal red cell. Successful applicants will join an active team consisting of seven MD's and PhD's in a recently funded NIH Program Project.

These candidates should forward their resume to: **Jiri Palek, MD, Department of Biomedical Research, St Elizabeth's Hospital of Boston, Tufts University School of Medicine, 736 Cambridge Street, Boston, MA 02135.** EOE. (NW579)A

RESEARCH CENTER OF CRETE
INSTITUTE OF MOLECULAR BIOLOGY AND BIOTECHNOLOGY

MAMMALLIAN MOLECULAR GENETICS GROUP
YEAST MOLECULAR GENETICS GROUP

2-YEAR POSTDOCTORAL POSITION
90,000 drachmas per month salary

Applications are invited for two postdoctoral positions funded by the Greek Secretariat of Research and Technology in the Institute of Molecular Biology and Biotechnology. The work in the mammalian molecular genetics group involves aspects of structure and expression of human glutamate dehydrogenase gene in normal and olivopontocerebellar artophy patients. The work in yeast group involves translational regulation of the GCN4 gene and studies on the mechanisms of transciptional activation by the GCN4 gene product. Applications should include a curriculum vitae and the names and addresses of three referees.

Send applications to: **Dr. Nicholas Moschonas** (Mammalian molecular genetics group), **Dr. George Thireos** (Yeast molecular genetics group), **Institute of Molecular Biology and Biotechnology, Research Center of Crete, PO Box 1527, 711 10 Haraklion, Crete, Greece.** (W4366)A

UNIVERSITÄT BASEL
MEDIZINISCHE FAKULTÄT

Infolge Berufung des bisherigen Amtsinhabers an eine andere Hochschule ist die Stelle des

Ordinarius für Pathologische Anatomie der Universität Basel und Vorstehers des Instituts für Pathologie am Kantonsspital Basel

neu zu besetzen.

Habilitierte in Pathologischer Anatomie sind gebeten, ihre schriftliche Bewerbung mit den üblichen Unterlagen bis 31 Oktober 1987 an das

Dekanat der Medizinischen Fakultät, Hebelstrasse 25, CH-4031 Basel, zu richten. (W4458)A

RESEARCH SCIENTISTS

Synergen, a leading biotechnology company in Boulder, Colorado, seeks recent PhD's with postdoctoral experience, technicians, and more senior people interested in becoming part of a rapidly expanding company. There are currently openings for the following persons:

Molecular Biologists to study the expression of eukaryotic genes encoding growth factors, neurotrophic factors, and proteins that modulate inflammatory processes. We also seek molecular biologists interested in developing high level expression systems in eukaryotes. **Protein Chemist** to participate in the isolation and characterization of proteins involved in tissue breakdown and repair, or in isolating and characterizing specific cell receptors. **Cell Biologists** to develop cell-based models of pathophysiological processes, to aid in the development of *in vitro* assays for bioactive proteins, and to study endothelial cell biology. **Biochemists** to define the mechanism of action of proteins interacting with membrane receptors and involved in signal transduction. Synergen offers competitive salaries, generous benefits, and the financial stability required for future development. Please send c.v., bibliography, and references to: **SYNERGEN, Recruitment Director, 1885 33rd Street, Boulder, CO 80301.** (NW593)A

Am **Pflanzenphysiologischen Institut und Botanischen Garten des Fachbereichs Biologie** der **Georg-August-Universität Göttingen**

ist die Stelle eines

Universitätsprofessors
(Bes.Gruppe C 3)

zum frühestmöglichen Zeitpunkt
zu besetzen

Dem/der Stelleninhaber/in obliegt die Ausbildung der Studierenden der Medizin im Biologiepraktikum (Teil Botanik). Außerdem ist eine Beteiligung an den Praktika des Pflanzenphysiologischen Instituts im Hauptstudium der Biologie erforderlich. In der Forschung soll der/die Bewerber/in eine modern Richtung der Pflazenphysiologie (z. B. Molekularbiologie) vertreten.

Die Einstellungsvoraussetzungen ergeben sich aus § 56 des Niedersächsischen Hochschulgesetzes (Nieders. GVBl. 1981 S. 263). Einzelheiten werden auf Anforderung erläutert (Tel.: 0551/39-7833 oder 22; Prof. G. Robinson, PhD).

Bewerbungen mit Lebenslauf, einer Darstellung des wissenschaftlichen Werdeganges, einschließlich der Lehrtätigkeit, und einem Schriftenverzeichnis werden bis zum **31. Oktober 1987** erbeten an den **Herrn Dekan des Fachbereichs Biologie der Georg-August-Universität Göttingen, Untere Karspüle 2, D-3400 Göttingen.**

NATIONAL INSTITUTES OF HEALTH

IMMUNOLOGIST/ MOLECULAR BIOLOGIST

Staff Fellow position for M.D., Ph.D., D.D.S., or equivalent with experience in expression libraries and construction of vectors to work on autoimmune diseases and/or transgenic mice. The candidate should have at least two years' postdoctoral experience. Starting salary range, depending on experience, is $24,000 to $46,000 per year. U.S. Citizenship required. The position is available immediately. Send curriculum vitae to: **Dr. Lois Salzman, Assistant Scientific Director, National Institute of Dental Research, National Institutes of Health, Building 30, Room 131, 9000 Rockville Pike, Bethesda, Maryland 20892. Tel: 301-496 1483.**

NIH is an Equal Opportunity Employer. (NW393)A

TECHNICAL DIRECTOR OF COLLABORATIVE RESEARCH IN CARBOHYDRATE SCIENCE

The University of Georgia/Department of Energy COMPLEX CARBOHYDRATE RESEARCH CENTER seeks a technical project director to coordinate collaborative research and service projects, monitor project progress, and fulfill reporting requirements. PhD in relevant scientific discipline, at least five years' experience in carbohydrate science, and demonstrated ability to supervise research projects required. Expected to build a support team of carbohydrate research technicians. Salary negotiable. Applications received by November 1, 1987 evaluated at that time. Late applications considered if position not filled. Send letter of application with complete c.v. to: **Dr Peter Albersheim or Dr Alan Darvill, Co-Directors, UGA/DOE Complex Carbohydrate Research Center, P.O. Box 5677, Russell Lab., Athens, GA 30613, USA.**

AA/EEO institution. (NW587)A

Two positions available for Ph.D.s with a strong background in molecular biology, biochemistry or protein chemistry.

Research projects under investigation include: gene regulation in teratoma cells, purification of factors binding to DNA control signal, and purification and characterization of liver growth stimulator inhibitor.

Send resume and a short summary of research experience to **Dr. Keiichi Itakura, Director, Dept. of Molecular Genetics, Beckman Research Institute of the City of Hope, 1450 East Duarte Road, Duarte, CA 91010-0269.** (NW606)A

FACULTY POSITIONS IN MICROBIOLOGY

The Department of Biological Sciences, Purdue University, is currently recruiting individuals with teaching and research expertise in three different areas: (i) eukaryotic microbiology, (ii) microbial pathogenesis, and (iii) microbial diversity. Preference will be given to candidates with research interests directed toward basic molecular mechanisms in the field of specialization. The positions will be 12-month, tenure-track appointments, and can be filled immediately at any rank. Interested individuals should send curriculum vitae, including brief description of research experience and plans, and lists of three references to: **Professor H.E. Umbarger, Chairman, Microbiology Search Committee, Department of Biological Sciences, Purdue University, West Lafayette IN 47907.** Applications should be submitted by 30 November 1987.

Purdue University is an Equal Opportunity/Affirmative Action Employer. (NW572)A

UNIVERSITY OF LEICESTER

Departments of Chemistry
and Pharmacology

POSTDOCTORAL RESEARCH ASSOCIATE IN INOSITOL PHOSPHATE RESEARCH

Applications are invited for a postdoctoral research associate supported by the SERC Molecular Recognition Initiative to join a collaborative group with Dr B V L Potter (Chemistry) and Professor S R Nahorski (Pharmacology) investigating intracellular recognition sites for inositol phosphate second messengers. Candidates should possess expertise in synthetic organic chemistry for the synthesis of novel analogues and must have an interest in involving themselves in ongoing investigations of the biological activity of such compounds.

The appointment, starting on 1st January 1988 at Research Support Grade 1A is for two years. The starting salary will be up to £9,865 per annum plus USS benefits, depending upon age and experience.

Applications with curriculum vitae and the names and addresses of two academic referees should be sent as soon as possible to Dr B V L Potter (Department of Chemistry) or Professor S R Nahorski (Department of Pharmacology), University of Leicester, University Road, Leicester LE1 7RH. (4196)A

UNIVERSITY OF LONDON

Royal Postgraduate Medical School
Department of Medicine
(Renal Unit)

RESEARCH OFFICER AND/OR TECHNICIAN

Two further posts will shortly become available in the Renal Unit, as part of a team on mechanisms in human and experimental glomerulonephritis. The Research Officer will be involved in studies of the effects of cytokines on gene expression and injury in the glomerulus, and the Technician in work on the genetics and regulation of experimental nephritis. Experience in immunochemical and/or molecular virological techniques is essential for the Research Officer and desirable for the Technician. Both posts are available for two years in the first instance.

Salary: Research Officer – £8,185 to £11,015 plus £1,393 London Allowance pa; Technician – £6,432 to £9,076 plus £1,201 London Allowance pa.

Application forms may be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0HS quoting reference AMB8.

Closing date: 15.10.87.

(4181)A

AFRC INSTITUTE OF ARABLE CROPS RESEAR

Rothamsted Experimental Stati
Harpenden Herts AL5 2JQ

PROTEIN CHEMIST/ MOLECULAR BIOLOGIS

Applications are invited for Higher Scientific Officer to w in the Biochemistry Departm on the isolation and characterizat of proteins that interact w regulatory sequences of cer seed protein genes. The ge encoding these "regulatory" p teins will then be isolated. T work forms part of a major pr ramme on the molecular biolo of cereal seed development. T post is for up to 3 years and supported by The Gatsby Four ation.

Qualifications: First or Upp Second Class Honours Degree Biochemistry or a related subj with at least two years releva postgraduate experience and interest in gene expression a DNA/protein interactions.

Salary on scale £9,2 £12,505. Non-contributory su annuation.

Applications, including f career details and the names two referees, should be sent to T Secretary, quoting Ref: 685 by October.

We are an equal opportuniti employer. (4178)A

UNIVERSITY OF NOTTINGHAM

Department of Applied
Biochemistry and Food Scienc

POSTDOCTORAL RESEARCH ASSISTA

A postdoctoral position te for two years is available to s the relationship between the str ture and function of an importa new polysaccharide system. T work is sponsored by a major foc manufacturer. The project w appeal to scientists interested working closely with an indust where there is a strong possibili that the results of their researc will have immediate application.

The research will involve t extraction of polysaccharides a their characterisation by a wi range of techniques probably in luding gel filtration, ORD, DS ultracentrifugation and rheologic methods. The most relevant di ciplines would be biochemistr chemistry or food science a previous experience with po saccharides would be desirable.

Salary on a scale £9,305 £11,015.

Applications including the nan and addresses of two refere should be sent to Dr J R Mitche Food Science Laboratories, Scho of Agriculture, Sutton Boningto Nr Loughborough, Leics LE 5RD. (4186)A

UNIVERSITY OF LONDON

Birkbeck College

POSTDOCTORAL RESEARCH ASSISTANT

Department of Crystallography

A vacancy exists for a Postdoctoral Research Assistant to work with Professor John Finney on computer simulation of water and specific ionic solutions under elevated conditions of temperature and pressure. This work is part of a recent contract awarded to the College by the Central Electricity Research Laboratories and will be tenable for three years only. Calculations will be made in a regime where the fluid begins to become compressible, where there are interesting and not straightforward problems for computer simulation. Applicants should therefore be experienced computer simulators. Salary will be within the range £12,408 to £13,428 pa, inclusive of London Weighting depending on age and experience.

Applications, including full CV and names and addresses of two referees, should be sent to: The Assistant Secretary (Personnel), (RO2/Cryst), (N), Birkbeck College, Malet Street, London WC1E 7HX.

Further details can be obtained from Professor John Finney (01-631 6138). (4206)A

KING'S COLLEGE LONDON (KQC)

Department of Biophysics

Applications are invited for two positions available now to join a small group working in the general area of the neuronal cytoskeleton and its environment in organelle motility.

1) A postdoctoral appointment funded by the SERC for three years to continue current work on the cloning of invertebrate neurofilament proteins from *Myxicola* and squid. Experience in cloning and recombinant DNA techniques is essential. Salary on scale 1A. (Commencing £10,440 + LA £1,393).

2) A research assistantship funded by the Wellcome Trust for two years to work on structural aspects of various neuronal proteins in normal and diseased nerves. Experience in image analysis, electron microscopy or computing would be a considerable advantage. Salary on the scale 1B (range £8,185 to £11,015 + LA).

Applicants interested in these positions should send as soon as possible (closing date 30 October 1987) a CV containing the names and addresses of two referees to: Dr P A M Eagles, Department of Biophysics, King's College London (KQC), 26-29 Drury Lane, London WC2B 5RL, from whom further details may be sought, 01-836 8851. (4180)A

THE QUEEN'S UNIVERSITY OF BELFAST

Department of Agricultural Botany

POSTGRADUATE/ POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited from agricultural botanists, botanists or other related agricultural scientists to work on a three-year MADD sponsored research project investigating genetic variation within the genus *Lolium*. The project will concentrate on the use of new physiological techniques to examine variation between cultivars in their resistance to stress, in particular winterhardiness and herbicide damage. A range of new morphological and physiological characteristics will be developed for introduction to distinguish between cultivars for the awarding of Plant Breeders Rights. The successful candidate may be given the opportunity of registering for a further degree on a part-time basis.

Salary range: £8,185 to £11,015 with superannuation under USS, initial placing dependent upon qualifications and experience.

Applications, quoting ref. 87/F, should submit a full curriculum vitae including the names and addresses of two referees to the Personnel Officer, The Queen's University of Belfast, Northern Ireland BT7 1NN. Closing date: 22 October 1987. (4200)A

UNIVERSITY OF ST ANDREWS

Department of Biochemistry and Microbiology

RESEARCH ASSISTANT VACANCIES

Applications are invited for two research assistant posts, one at Postdoctoral level and one at Postgraduate level.

The Postdoctoral post is funded by the SERC for 3 years to work on the molecular cloning of the barley nitrite reductase structural gene. Prior experience with recombinant DNA techniques would be an advantage but is not essential. Starting salary within range £9,305 to £11,015 per annum on the 1A scale.

The Postgraduate post is funded by the SERC for 18 months to work on the isolation and immunological characterisation of the plasma membrane of barley roots. Relevant experience would be an advantage but is not essential. Starting salary within range £8,185 to £8,735 per annum on the 1B scale.

Informal enquiries and applications with Curriculum Vitae and the names of two referees to: Dr J L Wray, Molecular Genetics Unit, Sir Harold Mitchell Building, University of St Andrews, St Andrews, Fife KY16 9TH.

(4202)A

RESEARCH ASSISTANT

BIOLOGICAL SCIENCES

A Research Assistantship, funded by the NAB is available in the Department of Biological Sciences. The subject of research is the formation of microbial biofilms in domestic and industrial water systems and the effects of biocides on these microorganisms.

Applications are invited from graduates in Microbiology, Biochemistry, Chemistry or any other suitable discipline. The successful candidate will be expected to register for a higher degree. The post is tenable initially for two years and may be extended into a third year.

Salary scale: £7,275 p.a. – £7,536 p.a – £7,797 p.a. including London Allowance of £1,215 p.a. (pay award pending). It is unlikely that the successful candidate will be appointed to the third point of that scale.

For further details and an application form please write on a postcard to the Deputy Personnel Officer (Recruitment), City of London Polytechnic, 117-119 Houndsditch, London EC3A 7BU quoting reference number 87/142. (4209)A

The Polytechnic is an equal opportunities employer

City Poly

We are positively committed to a policy of equal opportunity for all. We look forward to receiving suitable applications from all sections of the community which will be considered on merit, regardless of sex, age, race, ethnic origin, marital status, responsibility for dependants, sexual orientation or disability.

Department of Ecology and Evolutionary Biology University of Arizona

ECOLOGY & BEHAVIOR:

Applications are invited for two tenure-track positions in the general areas of behavior or ecology, including their evolutionary aspects. Applicants pursuing field or experimental studies are of particular interest. High originality in research and talent as a teacher will be important criteria used in selecting among applicants. These appointments will probably be made at the assistant professor rank. Applicants should mail a curriculum vitae, statement of research plans, reprints or preprints, and the names and addresses of at least three referees to **Conrad A. Istock, Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ, 85721.** The period for applications closes December 15, 1987. The University of Arizona is an equal opportunity/affirmative action employer. (NW599)A

UNIVERSITY OF CAMBRIDGE

Department of Pathology

HUMAN MOLECULAR GENETIC RESEARCH GROUP

TWO POSTDOCTORAL RESEARCH MOLECULAR BIOLOGISTS AND TWO RESEARCH TECHNICIANS (R6, R7)

Applications are invited for the above post to work on a long term programme, funded initially for five years by the MRC, to study the molecular genetics of the human Y chromosome and its relationship to the X. The work involves the identification, isolation and characterisation of human Y-borne genes including the testis determining factor, the H-Y gene, genes involved in spermatogenesis and a molecular analysis of the X-linked sterioid suphatase locus.

Postdoctoral applicants should have at least three years relevant experience in mammalian gene cloning and genome analysis. Salary on University age related scale.

Research Technicians should have HNC/Degree in relevant biological subject and have experience in molecular biology and/or cell culture.

Salary range R6 (7145-8275); R7 (7704-9359).

Applications in writing with names and addresses of two referees to

THE SUPERINTENDENT, DEPARTMENT OF PATHOLOGY,
TENNIS COURT ROAD, CAMBRIDGE CB2 1QP.

Please quote FS9905. (4227)A

UNIVERSITY OF SASKATCHEWAN

The Department of Anatomy, University of Saskatchewan, College of Medicine, invites applications for tenure-track position at the

ASSISTANT PROFESSOR

level. Applicants should have experience in teaching developmental biology to medical and science students as well as teratology and possibly neuroanatomy and/or gross anatomy. Background in molecular biology is desirable. Applicants should have proven research background and a strong research program. In accordance with Canadian immigration requirements, priority will be given to Canadian Citizens and permanent residents. Application, including curriculum vitae, names of three referees and an outline of proposed research should be submitted to: **Dr. Gary D. Burkholder, Head, Department of Anatomy, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, S7N 0W0.** (NW567)A

UNIVERSITY OF NOTTINGHAM

GRADE 4 RESEARCH TECHNICIAN

Applications are invited from men and women for the above post in the Department of Botany.

Main duties involve the preparation of tissue culture media and plant tissue cultures. Work includes the isolation, culture and fusion of protoplasts leading to fluorescence activated cell sorting and chromosomal and molecular analysis. Minimum qualification HNC, HTEC or equivalent.

Current salary scale £7,140 to £8,266 per annum for a 5 day, 37 hour working week.

This is a research funded post that will be initially offered on a fixed term contract not exceeding two years.

Apply in writing, enclosing full curriculum vitae and the names of two referees to: Professor E C Cocking, Department of Botany, University of Nottingham, University Park, Nottingham NG7 2RD. (4216)A

PUBLIC HEALTH LABORATORY SERVICE BOARD

CENTRAL PUBLIC HEALTH LABORATORY

Plasmid Section — National Collection of Type Cultures

SENIOR GRADE BIOCHEMIST/ MICROBIOLOGIST

Post-doctoral molecular biologist for 2 year EEC funded post (Biotechnology Action Programme) to develop the collections of gene probes, transposons and vectors, and to share in collection maintenance and the research of the Section.

The Section is responsible for a collection of plasmids, vectors, mobile genetic elements and host strains of medical importance, supplying such strains on request. Research interests include host/plasmid stability and the characterisation and epidemiology of plasmids and plasmid encoded genes.

Salary scale: £10,632-£13,896 per annum plus £718 London Weighting.

Further details from Dr V M Hughes, Ext 3738.

Please quote ref: NCTC/7.

Application form and job description from Personnel Department, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT.

Closing date: 23 October 1987.

UNIVERSITY COLLEGE AND MIDDLESEX SCHOOL OF MEDICINE

DEPARTMENT OF MEDICINE

POSTDOCTORAL RESEARCH ASSISTANT

required to work on the identification of the phosphate regulating gene which we have shown is on the short arm of the X-chromosome. This will involve the use of pulsed field electrophoresis and jumping libraries and will be done in collaboration with Dr K.E. Davies (Oxford). It is hoped that the successful candidate will have some previous experience in molecular biology techniques.

This position will be paid on scale IA-II according to experience, as part of a new five year programme grant from the Medical Research Council to study the Basis of Disorders of Mineral Metabolism.

Applications with the names and telephone numbers of two referees to Professor J.L.H. O'Riordan, Middlesex Hospital, London W1N 8AA. (4165)A

University of Zimbabwe

LECTURESHIP/ SENIOR LECTURESHIP/ ASSOCIATE PROFESSORSHIP IN DEPARTMENT OF BIOCHEMISTRY

Applicants should have a postgraduate degree, preferably a PhD in a speciality in Nutrition and with a strong background in Biochemistry. University teaching experience would be an advantage. The successful candidate will be expected to (i) teach short courses in nutritional biochemistry to science, medical and veterinary students (ii) develop and organise full nutrition courses at University level (iii) initiate a programme of research in his/her area of interest (iv) supervise postgraduate students.

Salary Scale

Medical (including professional Supplement) — Lecturer Z$22,224–Z$30,840, Senior Lecturer Z$31,740–Z$33,960, Associate Professor Z$34,884–Z$35,640.

Non-Medical — Lecturer Z$18,216–Z$26,832, Senior Lecturer Z$27,732–Z$30,360, Associate Professor Z$31,284–Z$32,640. Appointment on the above scales according to qualifications and experience.

Conditions of Service: Both permanent and short-term contracts are offered. Persons who are not Zimbabwean citizens may be appointed only on a short-term contract basis for an initial period of two years. Short-term contracts may be extended.

Six copies of applications giving full personal particulars which should include full name, place and date of birth, qualifications, employment and experience, present salary, date of availability, telephone number and names and addresses of three referees should be addressed to the Deputy Registrar (Administration), University of Zimbabwe, PO Box MP 167 Mount Pleasant, Harare, Zimbabwe (Telex 4-152 ZW).

Applicants in the UK should also send an additional copy to the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom further particulars are available.

Closing date for receipt of the applications is 30 October 1987. (W4470)A

UNIVERSITY OF WARWICK

RESEARCH TECHNICIAN

In the Plant Biochemistry Research group to work on the molecular biology of protein transport into chloroplasts.

The post is industrially funded and available for 2 years.

Applications are invited from

tions and experience in biochemistry, and/or molecular biology.

Salary on Technician Grade 3 scale: £6,314—£7,448 pa.

Application forms from the Personnel Office, University of Warwick, Coventry CV4 7AL (0203 523685) quoting Ref No. 7/T/87, (please mark clearly on envelope). Closing date 15 October 1987. An Equal Opportunities Employer. (4166)A

UNIVERSITY OF WALES COLLEGE OF MEDICINE

Department of Child Health

Applications are invited for th following two research posts funde by the Wellcome Trust for thre years. The research group is in volved with the investigation c disorders of sexual differentiatio in humans.

Post 1:

POSTDOCTORAL RESEARCH OFFICER

(University Research and Analogous Staff Grade 1A) required t study androgen receptor bindin and action in genital skin fibro blasts from patients with syn dromes of androgen insensitivity The appointee will use molecula biology techniques to complemen ongoing biochemical studies o steroid hormone action. Relevan experience in molecular biolog and tissue culture would be a advantage. Technical assistance i available. There will be close collaboration with the Molecula Biochemistry Unit at the Tenov Institute for Cancer Resear Commencing salary up to £11,01 per annum depending on age.

Post 2:

RESEARCH TECHNICIAN

(University Technician Grade 4) required to assist with studies of the molecular genetics of 21-hydroxylase enzyme deficiencies. The project involves analysis of DNA from families with congenital adrenal hyperplasia together with HLA and complement typing. The appointee will also assist with studies of the androgen receptor in genital skin fibroblasts as part of the general research programme. Commencing salary up to £7,786 per annum.

Candidates for both posts should send a detailed curriculum vitae stating the post in which they are interested and quoting ref nc C10/74 together with the names and addresses of three referees to: The Registrar and Secretary (Personnel Office), University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, to arrive within three weeks of the appearance of this advertisement.

Further information can be obtained either from Dr I A Hughes on 0222-755944 Ext 2274 or Dr Bronwen Evans on Ext 3135. (4192)A

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nature

Please mention
when replying to
these advertisements

ROYAL POSTGRADUATE MEDICAL SCHOOL

(University of London)

DEPARTMENT
OF CHEMICAL PATHOLOGY

TECHNICIAN

required for a research project on the cardiovascular neuropeptide, CGRP. An extensive programme of research, funded by the British Heart Foundation, into the role of CGRP in the cardiovascular physiology and pharmacology of CGRP is being undertaken jointly by Professor I. MacIntyre, RPMS, and Professor H.R. Morris, Imperial College. The technician would be concerned with the development and application of more specific and sensitive CGRP assays suitable for clinical and experimental use.

Candidates should have HNC or a degree and relevant experience.

Salary on scale £6,432—£7,761 plus £1,201 London Allowance a year.

Application forms and further particulars may be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0HS quoting reference ACR2.

Closing date: 15.10.87.

(4164)A

UNIVERSITY OF BIRMINGHAM

Department of Biochemistry

LECTURESHIP IN BIOCHEMISTRY

A Lectureship has been established on Health Authority funds to maintain and develop links between this academic Department and the Liver Unit at the Queen Elizabeth Hospital, which is one of only two nationally-funded centres for liver transplantation and for the treatment of patients with fulminant hepatic failure. The post will be available for five years in the first instance and the appointment may be made on the Lecturer 'A' or 'B' scale, in ange £8,735 to £18,210. The ippointee, who will be able to ollow his/her own line of research vithin the overall direction of the Jnit, will have certain laboratory upervisory duties and opportuniies for some teaching of students. or further particulars, telephone 21 472 1301 ext 2559, quoting reference number B4561. No fornal application form. Three opies of application, including ull Curriculum Vitae and naming hree referees to Assistant Registrar (Science), PO Box 363, Biringham, B15 2TT by 23 October 987.

An Equal Opportunities
Employer

(4220)A

RIVERSIDE HEALTH AUTHORITY

Westminster Hospital
Melanoma Research Fund Appeal

TECHNICIAN/RESEARCH OFFICER

Required at Westminster Hospital to assist in the development and day to day running of a tissue culture laboratory attached to the Medical Oncology Unit. The work will involve evaluation of experimental and established drugs with different tissue culture techniques in relation to clinical trials conducted in the Unit. Duties to commence as soon as possible.

Salary in the range of £8,020 to £12,870 plus London Weighting.

For job description and application form please ring 01-400 0296 (24 hours); to be returned by Friday 16th October 1987 with curriculum vitae and the names of two referees to: Mr Mark Hirst, Unit Personnel Department, Queen Mary's Nurses Home, 20 Page Street, London SW1P 4AP. (4189)A

THE UNIVERSITY OF SYDNEY

CHAIR OF PHYSIOLOGY

Reference No. 38/18

Applications are invited for a Chair of Psysiology which will become vacant upon the retirement of Professor W Burke at the end of 1987.

Salary: A$58,870 per annum. There is provision for a preclinical loading of A$5,223 per annum where appropriate.

Applications, in duplicate, quoting reference no., including curriculum vitae, list of publications and the names and addresses of three referees to the Registrar, University of Sydney, NSW 2006, Australia by 30 November 1987.

The University reserves the right not to proceed with the appointment for financial or other reasons.

Further information is available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (W4456)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Centre for Information Science Research

Applications are invited for appointment to the following positions available in the Centre for Information Science Research from 1988:

(1) Postdoctoral Fellow/ Research Fellow (Computation using Neuronal Networks)

A position is available for an applicant with a PhD in engineering or science to investigate the use of self-organising neuronal-like networks for feature extraction from poorly defined data. Applicants should have a strong background in mathematics and computation and an enthusiasm for investigating uses of parallel processing with adjustable connectivity. No detailed knowledge of the nervous system is required. The successful applicant will work with Dr S Redman of the Experimental Neurology Unit, John Curtain School of Medical Research. Further information from Dr Redman (tel. (062) 492602. Ref IS.17.9.1.

(2) Postdoctoral Fellow/ Research Fellow

A position is available for an applicant with a PhD in either physics, computer science, visual sciences or a related area and with a preference for interdisciplinary research. The successful applicant will model and construct a mechanism for artificial vision based upon natural vision systems under the direction of Professor G A Horridge of the Centre for Visual Science, the Research School of Biological Sciences. Applicants should have a good knowledge of software for modelling the system and sufficient experience to construct a real system with either analogue or digital circuitry. Further information from Professor Horridge, tel. (062) 494532. Ref IS.17.9.2.

Closing date for both positions: 30 October 1987.

Salary: Postdoctoral Fellow Grade I (fixed point) A$24,535–A$28,029 pa; Research Fellow A$28,381–A$37,122 pa. Appointment: Research Fellow up to three years, possibility of extension to five years; Postdoctoral Fellow normally two years, possibility of extension to three years. Enquiries welcomed from persons interested in secondment from their home institutions.

Applications should be submitted in duplicate to the Registrar, The Australian National University, GPO Box 4, Canberra ACT 2601, Australia, quoting reference number and including curriculum vitae, list of publications and names of at least three referees. The University reserves the right not to make an appointment or to appoint by invitation at any time.

Further information is available from the Registrar or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

The University is an equal

Coleg Prifysgol Cymru
Aberystwyth
The University College of Wales

Department of Botany & Microbiology

POSTDOCTORAL PROTEIN BIOCHEMIST: MOLECULAR PLANT PATHOLOGY

Required to work on the isolation of a factor involved in the host/pathogen interaction in the Witches' Broom fungal disease of cocoa.

Candidates with a PhD and experience in protein purification in any area of biology will be considered, but should be willing to develop an interest in the expanding field of molecular plant pathology.

The project is co-ordinated by Drs J N Hedger and I M Scott and is funded on an annual basis by The Biscuit, Cake, Chocolate and Confectionary Alliance. Salary will be on the RA 1A Scale (minimum starting salary £9,305). Curriculum vitae and names of two referees should be sent to the **Staffing Office, Old College, King Street, Aberystwyth, Dyfed SY23 2AX, not later than the 21st October 1987.** Further details can be obtained from **Drs Hedger and Scott on Aberystwyth (0970) 3111, ext 3065/3081.** (4172)A

CENTRE DE LA RECHERCHE SCIENTIFIQUE (CNRS), PARIS

POSTDOCTORAL POSITION IN BIORGANIC CHEMISTRY

CNRS funded position available: laboratory studying bacterial enzyme inhibitors. A recent PhD degree with strong background in biochemistry and good knowledge in organic chemistry required. Experience in protein purification and characterisation and in chemical synthesis highly appreciated.

Send résumé and three referees letters to: Professor François Le Goffic, CNRS-Cercoa, 2 à 8, rue Henri Dunant, 94320 Thiais, France. (W4464)A

NEUROBIOLOGIST

A position is available in the Department of Neurobiology and Behaviour, State University of New York at Stony Brook, for a postdoctoral fellow to collaborate in studies of retinal neurons, including photoreceptors, using patch-clamp techniques. Send curriculum vitae and a brief statement of research experience and interests to: **Dr. Gary G. Matthews, Abteilung Membranbiophysik, Max-Plaack-Institut für biophysikalische Chemie, Postfach 2841, D-3400 Göttingen, Federal Republic of Germany.** SUNY Stony Brook is an affirmative action/equal opportunity educator and employer. AK#182.

(NW581)A

POSTDOCTORAL POSITION

available immediately to produce monoclonal antibodies to cytoskeletal proteins and biological growth factors that regulate hematopoietic differentiation. Background in immunology, immunochemistry and monoclonal antibody technology is desired.

Projects include indentification and purification of differentiation-associated regulators and cytoskeletal components.

Send CV and names of two references to:

Dr. Nicholas Dainiak, Chief, Division of Clinical Research, St. Elizabeth's Hospital, Boston, MA 02135, (617) 789-2733. EOE. (NW578)A

POST-DOCTORAL FELLOW POSITION

Research on regulation of dynein and flagellar motility by cAMP, Ca^{2+}-calmodulin & protein phorylation/dephosphorylation. Availability immediately; for up to 3 years. Salary depending on experience. Send c.v. and 3 letters of reference to: **Dr. Joe Tash, Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030.**

An Affirmative Action/ Equal Opportunity Employer (NW605)A

THE NATIONAL HOSPITAL FOR NERVOUS DISEASES

Queen Square, London WC1N 3BG

SENIOR SCIENTIST

Required from 1st November 1987 for a new post in a recently established laboratory (in the University Department of Clinical Neurology, Institute of Neurology) which will provide genetic services using linked DNA markers to families with inherited neurological disorders, particularly Huntington's disease. Candidates should have a PhD and must have experience in molecular genetic techniques; experience in genetic linkage studies is desirable.

Salary scale £10,632 to £13,896 plus £1,201 London Weighting Allowance.

Further information obtainable from the Personnel Department, National Hospital for Nervous Diseases, Queen Square, London WC1N 3BG, to whom curriculum vitae should be sent within two weeks of the date of this advertisement. Informal enquiries to Dr A Harding, telephone 01-837 3611 ext. 4262. (4188)A

McMASTER UNIVERSITY

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL POSITION

Available to study sorting and targeting of enveloped virus (HSV and Bunya) (P.N.A.S. 84, 5675 1987) membrane glycoproteins and reverse transcriptase structure-function using cloned gene and site-directed mutagenesis and retrovirus mediated gene transfer. Starting salary $20,000-$24,000. Opportunity for limited teaching. Starting date November 1st. Please send curriculum vitae and three letters of reference to: **Professor H.P. Ghosh, Chairman, Department of Biochemistry, McMaster University, Hamilton, Ontario, L8N 3Z5, Canada.**

UNIVERSITY OF LEICESTER

Department of Medicine

The University Department of Medicine which is based at the Clinical Sciences Building at Leicester Royal Infirmary, requires a

GRADE 3 TECHNICIAN

to assist with research into the causes and treatment of high blood pressure. This is an established post with effect from 1st August 1987.

A minimum of 3 years' previous laboratory experience in a biological or medical sciences department is essential and applicants should be qualified to ONC standard or its equivalent. Salary scale £6,314 to £7,448 a year depending upon qualifications and experience.

Applications (including a curriculum vitae), in writing, should be sent to Mrs J M Brice, Chief Technician, Department of Medicine, Clinical Sciences Building, Leicester Royal Infirmary, PO Box 65, Leicester LE2 7LX, as

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UNIVERSITY OF NEWCASTLE UPON TYNE

Department of Biochemistry

JUNIOR RESEARCH ASSOCIATE

Applications are invited from Honours Graduates in Biochemistry or a related subject for this MRC funded post available for one year and starting as soon as possible to work on the mechanisms of hormonal regulation of lipotrotein lipase gene expression. Experience in molecular biology would be an advantage, but is not essential.

Starting salary will be up to £9,865 p.a. on the Range IB salary scale: £8,185-£11,015 according to qualifications and experience.

Applications including a full cv (3 copies) and the names and addresses of 3 referees to Dr. Megs Rogers (from whom further information may be obtained), Department of Biochemistry, Ridley Building, The University, Newcastle upon Tyne, NE1 7RU by 16th October 1987. (4218)A

UNIVERSITY COLLEGE LONDON

Department of Physics and Astronomy

LECTURER IN PHYSICS

Applications are invited for a lectureship in the Department o Physics and Astronomy from October 1988. The successful candidate will probably be under th age of 35 and will be expected t undertake research in positro physics as part of a strong existin group in the field. Experience i positron research would be a distinct advantage but any experimental physicist of proven ability and clear potential who is willing to enter the field, will be considered. The starting salary will be on the Lecturer A scale, ie £9,260–£14,500 pa (1 March 1988 scale) + £1,393 LA, with possible promotion to Lecturer B later on.

Applications including the names of three referees should be sent to the Senior Assistant Secretary (Personnel), University College London, Gower Street, London WC1E 6BT before [illegible] October 1987. (4222)A

UNIVERSITY OF BIRMINGHAM

Department of Cancer Studies

Applications invited for the post of

RESEARCH FELLOW

availabe from 1 October 1987 and funded for five years by CRC. The Research Fellow will join a research group studying the role of viruses in the aetiology of cervical neoplasia. Experience in molecular or virological techniques would be an advantage. Salary is on scale: Research Fellow 1A £9,305 t £14,825. Maximum starting £12,03 with superannuation.

Informal enquiries from Dr L S Young on 021-472 7607. Applications (3 copies) to Assistant Registrar, The Medical School, University of Birmingham, Birmingham B15 2TJ from whom further particulars are available by 23 October 1987. Quote reference RF/CS/LSY.

An Equal Opportunities Employer. (4193)A

ROYAL POSTGRADUATE MEDICAL SCHOOL

(University of London)

CARDIOVASCULAR RESEARCH UNIT

RESEARCH ASSISTANT

Graduates, or equivalent, in physiology or pharmacology or related fields are invited to apply for the post of research assistant in the Cardiovascular Research Unit.

The postholder will be responsible for conducting *in vitro* experiments in vascular tissue and assist with cell culture, as part of a study of the control of the coronary vasculature. The post is available for a three year period and suitable candidates will have the opportunity to register for a higher degree.

Salary on scale £5,900—£9,076 plus £1,201 London Allowance a year.

Application forms may be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0HS quoting reference JT/RA.

Closing date: 23.10.87.

UNIVERSITY OF CAMBRIDGE

Department of Clinical Veterinary Medicine

POST-DOCTORAL RESEARCH ASSISTANT/ASSOCIATE

Applications are invited for three year appointment funded b the AFRC to assist the Professo of Animal Welfare on "Welfar indicator evaluation and th assessment of welfare in dr sows". Candidates should have PhD on a relevant behavioural physiological or agricultural topi and be not more than twenty seve years of age.

The salary for a Research Associate is £11,015, and for Research Assistant it will be on th age related scale.

Applications, curriculum [illegible] and the names of not more tha two referees should be sent in triplicate to Professor D M Broom Department of Clinical Veterinar Medicine, Madingley Road Cambridge CB3 0ES from who further particulars can be obtained, to reach him not later tha 26 October 1987. There is n

UNIVERSITY OF NOTTINGHAM

Department of Physics

ELECTRONICS ENGINEER

ıpplications are invited for the emporary post of Experimental)fficer with special expertise in igital and/or RF electronics to vork on the design and construction f a novel nuclear magnetic resonnce microscope to operate at 500 1Hz. The product is supported by Royal Society Paul Instrument 'und grant for a period of up to ıree years. Applicants should ave a degree or equivalent in lectronic engineering; experience ı nuclear magnetic resonance 'ould be an advantage.

The planned microscope will be unique instrument capable of udying a whole range of problems ı biology and materials science.

The salary will be in the range of 10,440 to £11,460 per annum ınder review) plus USS.

Applicants should write to: rofessor P Mansfield, University Nottingham, University Park, ıngham NG7 2RD, enclosing V and the names of at least two ferees. The closing date for app:ations is Monday 19th October)87. (4215)A

FELLOWSHIPS

INIVERSITY COLLEGE OF IORTH WALES, BANGOR

EPARTMENT OF BIOCHEMISTRY & SOIL SCIENCE

OSTDOCTORAL FELLOW

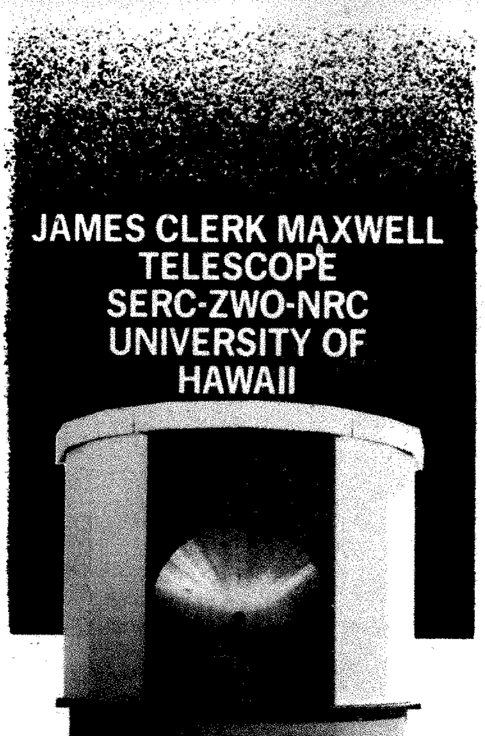
ary Range £9,305—£11,015 (next review date 1.3.88)

pplications are invited from suitıly qualified biochemists or olecular biologists to join a oup investigating the biochemıl basis of schizophrenia. Individuals experienced in membranes ıd receptors, molecular genetics cell culture are particularly ıcouraged to apply. The study is collaboration with the Gwynedd istrict General Hospital, ıngor.

The post, to be known as the ırbara Dorothy Worlidge llowship, is funded by the hizophrenia Association of eat Britain for 3 years starting mediately.

Applications (two copies), ing details of age, education d experience together with the es and addresses of two ferees should be sent to the affing Officer, University ollege of North Wales, Bangor, wynedd LL57 2DG, from whom rther particulars may be tained.

Enquiries to Professor J.W. yne (Tel: 0248 351151, ext. 50) (4175)E



JAMES CLERK MAXWELL TELESCOPE SERC-ZWO-NRC UNIVERSITY OF HAWAII

Senior/Principal Research Fellow in Millimetre & Submillimetre Astronomy

As part of the collaboration between the United Kingdom Science and Engineering Research Council (SERC), the Netherlands Organisation for the Advancement of Pure Research (ZWO), the Canadian National Research Council (NRC) and the University of Hawaii in millimetre and submillimetre astronomy, there is a vacancy for a Senior/Principal Research Fellow in the areas of millimetre and submillimetre astronomy. The successful applicant will be expected to undertake research with the James Clerk Maxwell Telescope which was recently opened on the summit of Mauna Kea. The operational phase of the Telescope began on 1 August 1987, although for an initial period, there will remain a considerable amount of commissioning of the facility to be undertaken. The Research Fellow will be primarily based at the University of Hawaii's Institute for Astronomy which is located on the Honolulu campus of the University. It is expected that the successful applicant will spend a considerable fraction of his/her time working with the team of UK/Netherlands/Canadian scientists based at the Hawaiian Headquarters of the JCMT operation which is located on the campus of the University of Hawaii in Hilo.

The successful applicant will enjoy all the privileges of being a staff member of the Institute for Astronomy, including access to the other telescopes on Mauna Kea on the same basis as staff members of the University of Hawaii, although it is expected that the major emphasis of the research of the successful applicant will be with the JCMT. The succcessful applicant may be required to supervise the work of research students who may undertake part or all of their studies in millimetre and submillimetre astronomy at the Institute for Astronomy in Hawaii.

The duration of the Fellowship is somewhat flexible subject to a minimum of one year and a maximum of three years.

The stipend is in the range of £11,538 to £12,975 (SRF) p.a., £14,217 to £18,070 (PRF) p.a. dependent on age and length of relevant experience. In addition overseas allowances (linked to stipend and marital status) ranging from £4,300 p.a. to £9,400 p.a. may also be payable.

Scientists who hold a PhD and already have a strong record of successful post-doctoral research in millimetre, submillimetre or a closely related field are invited to apply. Applications in the form of a curriculum vitae and a proposed programme of research are required by 30 October 1987 and should be addressed to:

The Director, Professor M. S. Longair,
Royal Observatory, Blackford Hill,
Edinburgh EH9 3HJ, Scotland, UK.
Telephone: 031-667 3321. Telex: 72383. (4228)E

UNIVERSITY OF LONDON

The School of Pharmacy

MRC POSTDOCTORAL FELLOWSHIP IN PHARMACOLOGY

This is a three year postdoctoral research fellowship funded by a MRC grant awarded to Prof N G Bowery. The research project will concern the autoradiographic analysis of receptors for the inhibitory amino acid GABA in mammalian brain. Experience of receptor autoradiography would be an advantage but not essential.

Starting salary £10,698 inclusive of London Allowance.

Applications in the form of a curriculum vitae and naming two referees should be submitted to: Mr N J Rampley, Assistant Secretary, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX (4190)E

RESEARCH FELLOWSHIP

GIRTON COLLEGE CAMBRIDGE

Applications are invited for a scientific Research Fellowship open to graduates of any university and tenable for three years from 1 October 1988. The applications should be in the fields of Mathematics, Natural Sciences, Geography and allied subjects.

The emoluments of the Fellowship are reviewed annually. From 1 October 1987 the stipend for a person who has not completed a Ph.D. is £5000 per annum; for a person with a Ph.D. £7550 per annum rising by two increments to £7750 per annum. Financial support from other sources may be taken into account.

Fellowships are pensionable under USS. Residence is provided, if required; a charge of £74.40p. month is made towards residence, and Commons (i.e. meals) are free of charge. If the Research Fellow is married, a living out allowance of £892p.a. and child allowance of £250 per annum for the first chid is made. Research Fellows will, if they wish, be permitted to teach up to six hours per week.

Applications (2 copies) on a form which may be obtained from the **Secretary to the Council, Girton College, Cambridge CB3 0JG**, must be returned to her not later than **5 November 1987**. A statement of approximately 1000 words outlining the work candidates wish to submit in support of their applications and the research they propose to do if elected will be required at the same time as the application is returned. The applicant should also include a short resume of his/her research for an informed layman. If the applicant is short-listed he/she will be asked to submit work in support of the application; it is suggested that the work to be submitted should be a dissertation and/or other writings.

Since the College is advertising for a Research Fellow in the Arts as well the Science applicants should state when writing for an application form whether their subject of research is an Arts or a Science subject. (4176)E

EMBL

European Molecular Biology Laboratory

PhD Programme in Molecular, Cellular and Structural Biology

The European Molecular Biology Laboratory will award a small number of predoctoral fellowships for graduates wishing to study for a Ph.D. at EMBL, Heidelberg. Areas of study include: **Cell Biology** (protein and membrane traffic, membrane biogenesis, posttranslational modifications, functional aspects of the cytoskeleton), **Differentiation** (oncogenes, growth factors and growth control, haemopoetic cell differentiation, developmental and tissue-specific gene expression); **Gene Structure and Regulation** (regulation of mammalian and viral gene expression, protein engineering, structure and function of small RNAs); **Biological Structures** (two and three dimensional analyses of biological structures using X-ray crystallography and electron microscopic techniques) and **Biocomputing** (theory and analysis of protein structure and genomic sequence information, development of computer methods for molecular biology).

To be eligible, candidates must hold, or anticipate receiving in 1988, a first or upper second class honours degree (Great Britain), a masters degree or a diploma in biological sciences, physical sciences or mathematics.

Candidates must be citizens of the member states of EMBL or EMBC namely: Austria, Belgium, Denmark, Finland, France, Federal Republic of Germany, Greece, Iceland, Ireland, Israel, Italy, Netherlands, Norway, Spain, Sweden, Switzerland or the United Kingdom.

Short-listed candidates will be asked to visit EMBL (travel expenses reimbursed) for an interview. Those awarded predoctoral fellowships will be expected to begin working at EMBL in September 1988. The normal duration of the fellowships will be 3 years subject to annual review.

The closing date for completed applications is December 15, 1987. Successful candidates will be notified by the end of March 1988.

Application forms and a catalogue giving further information about the research at EMBL and the groups participating in the Laboratory's predoctoral programme is available on request from: **Dean of Graduate Studies, EMBL, Postfach 10.2209, 6900 Heidelberg, West Germany.** (W4379)E

UNIVERSITY OF EDINBURGH

Department of Pathology

POSTDOCTORAL RESEARCH FELLOWSHIP IN CANCER STUDIES

Applications are invited for two Postdoctoral Research Fellowships funded for 3 years by the Cancer Research Campaign.

One Fellowship (directed by Professor C C Bird and Dr A H Wyllie) will be concerned with the genetic basis of human colorectal cancer and its evolution in familial and sporadic forms of disease. The role of inducible oncogenes in determining tumour aggressiveness in vitro and in vivo will also be studied. Applicants should have experience of molecular and/or cell biology.

by Dr M L Hooper) is concerned with the production of chimaeras from mouse embryonal stem cells subjected to targeted mutagenesis, in order to introduce chosen mutations into the mouse germ line. The ultimate aims include the development of mouse models for human familial cancers. Previous experience in the production of mouse chimaeras is desirable.

Appointments will be made on the R/A 1A scale (£9,305 to £14,825) according to qualifications and experience. Further information can be obtained from Professor C C Bird, Department of Pathology, University Medical School, Teviot Place, Edinburgh EH8 9AG (031-667 1011 Ext. 2227) to whom applications including a curriculum vitae and the names and addresses of two referees should be sent by 31st October 1987. Please quote reference no.

POSTDOCTORAL FELLOWSHIPS IN THEORETICAL PHYSICS AND ASTROPHYSICS

Several Fellowships will be awarded for postdoctoral study in theoretical physics and theoretical astrophysics at the California Institute of Technology. These Fellowships, which begin in Fall 1988, are two- or three-year duration, carry annual stipends of $30,000 and, in addition, offer a research-expense fund of $3,000 per year. A Richard Chase Tolman Fellowship and a Division Research Fellowship will be awarded on the basis of an international competition, and a Bantrell Fellowship will be awarded to a U.S. or an Israeli citizen.

This Fellowship program has been established to offer scientists, typically within two years of receipt of the Ph.D., the best possible opportunity to develop their talents. Research will be encouraged in areas in which Caltech's faculty is currently active:

Astrophysics
Computational Physics
Condensed Matter Physics
Elementary Particle Physics
Gravitational Physics
Mathematical Physics
Nuclear Physics

Materials in support of an application or nomination should be sent to Professor H. David Politzer, Executive Officer for Physics, c/o Marge D'Elia, Mail Code 103-33, California Institute of Technology, Pasadena, CA 91125. These materials should include curriculum vitae, bibliography of publications and preprints of manuscripts not yet published, and a description of the anticipated research program. The candidate or nominator is requested to ensure that at least three letters of recommendation be sent directly to Caltech.

ALL MATERIALS SHOULD ARRIVE AT CALTECH BEFORE DECEMBER 1, 1987

Fellowship candidates will automatically be considered for other available postdoctoral positions at Caltechn in their fields of interest.

Caltech is an Affirmative Action/Equal Opportunity Employer, Women/minorities are encouraged to apply. (NW601)E

FELLOWSHIP THORACIC ONCOLOGY

A position is available (starting January 1988) in the Department of Thoracic Surgery and Oncology at the Roswell Park Memorial Institute, Buffalo, NY.

The core of the training will be centered around diagnosis, treatment, and research in the intrathoracic malignancies, especially lung cancer. Areas of interest include chemotherapy, immunotherapy, research in lung cancer immunology, and lung transplantation.

The fellow must have had at least 3 years of approved residency training in the U.S.

Interested candidate should contact: **H. Takita, M.D., D.Sc., F.A.C.S., Chief, Department of Thoracic Surgery and Oncology, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, NY 14263; Telephone: 716-845-2300.** (NW574)A

EMBO

EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

SHORT TERM FELLOWSHIPS

in molecular biology

The European Molecular Biology Organization awards, to scientists working in Europe and Israel in the field of molecular biology and allied disciplines, short term fellowships of one week up to three months duration. The fellowships are to support collaborative research between laboratories in different countries and provide a travel grant and subsistence allowance. Applications may be made at any time and are decided upon soon after the receipt of application.

Applications for exchanges between laboratories within any one country cannot be considered. Fellowships involving transatlantic travel are awarded only in extremely exceptional circumstances. Inquiries should be accompanied by a self-addressed adhesive label.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022. 40, F.R.G. (W3679)E

University of Colorado

VISITING FACULTY AND POSTDOCTORAL FELLOWSHIPS (1988-89)

FOR RESEARCH IN

- **atmospheric chemistry**
- **analytical chemistry**
- **environmental chemistry, biochemistry and geochemistry**
- **atmospheric and climate dynamics**
- **remote sensing**
- **global change**

at the Cooperative Institute for Research in Environmental Sciences (CIRES).

Five competitive fellowships will be awarded to recent Ph.D. recipients and to senior scientists, including faculty on sabbatical leave. Stipends average $26,500 for twelve months. Appointments can begin at any time during the year. Selection of awardees is made in part on the likelihood of active interaction with CIRES scientists and other research groups in Boulder. Senior CIRES-affiliated scientists working in these fields include James Avery, Susan Avery, Ben Balsley, Roger Barry, John Birks, Henry Diaz, R. Ray Fall, Lang Farmer, Fred Fehsenfeld, Alexander Goetz, Howard Hanson, William Hay, Murray Johnston, Bruce Koel, Eric Kraus, Uwe Radok, Colin Ramage, George Reid, Robert Sani, Russ Schnell, Robert Sievers, and Harold Walton. Candidates are encouraged to contact one or more of the senior investigators as they are preparing their proposals. Apply to

PROF. R. SIEVERS, Director, CIRES,
Visiting Fellows Program
University of Colorado, Campus Box 449
Boulder, CO 80309

Include vitae, publications list, and brief outline of proposed research. Also request that three persons familiar with your qualifications send letters of recommendation. Junior applicants must submit undergraduate and graduate transcripts. First consideration will be given to applications received by December 1. Final application deadline is February 15.

The University of Colorado at Boulder has a strong institutional commitment to the principle of diversity. In that spirit, we are particularly interested in receiving applications from a broad spectrum of people, including women, members of ethnic minorities and disabled individuals.

(NW604)E

MILLIKAN POSTDOCTORAL FELLOWSHIP IN EXPERIMENTAL PHYSICS OR ASTROPHYSICS

Caltech's Division of Physics, Mathematics and Astronomy offers a Millikan Fellowship for postdoctoral study in experimental physics or experimental astrophysics at the California Institute of Technology. This Fellowship will have a two- or three-year duration, beginning in Fall 1988, and will carry a stipend of $30,000 per year. In addition, the Fellow will have access to a research-expense fund of $2000 per year.

The Millikan Fellowship Program has been established to offer junior scientists the opportunity of pursuing new and innovative experimental research. It is expected that this research will require the support and use of facilities of one of the established experimental groups at Caltech. Currently, Caltech has groups working in:

Astronomical Detector Development
Gravitational Physics
High Energy Astrophysics
High Energy Physics
Infrared and Submillimeter Astronomy
Low Energy Particle Physics
Low Temperature Physics
Neurobiology
Nuclear Physics
Radio Astronomy
Surface Physics

The application should indicate the group with which the candidate would most likely be associated and discuss particular areas of interest.

Candidates may either apply directly, or they may be nominated by colleagues or professors. Materials in support of an application or nomination should be sent to Professor E.C. Stone, Chairman, Division of Physics, Mathematics and Astronomy, 103-33, California Institute of Technology, Pasadena, CA 91125. These materials should include curriculum vitae, bibliography of publications and preprints of manuscripts not yet published, and a description of the anticipated research program. The candidate or nominator is requested to ensure that at least three letters of recommendation be sent directly to Caltech.

ALL MATERIALS SHOULD ARRIVE AT CALTECH BEFORE JANUARY 1, 1988

Fellowship candidates will automatically be considered for other available postdoctoral positions at Caltech in their fields of interest.

Caltech is an Affirmative Action/Equal Opportunity Employer. Women/minorities are encouraged to apply.

(NW595)E

LINCOLN COLLEGE

iversity College of Agriculture
New Zealand

POSTDOCTORAL FELLOWSHIPS

pplications are invited for two ostdoctoral Fellowships, one in iochemistry and the other in Soil cience. The Fellowships are unded by the University Grants ommittee for a term of two years, o be taken up before July 1989.

Applicants should be no more han 35 years of age and have been warded or qualified for the award f the degree of Doctor of Philoophy.

The Emolument for these ellowships shall be at the first ep of the New Zealand Univerties Lecturer scale, currently $32,000 per annum.

The Fellowships will involve:

BIOCHEMISTRY

(Vacancy 87/50)

wo lines of lambs with different sulin status have been genetically lected at the College. The long m objective of the programme volves locating the insulin gene and the sequences upstream from the insulin gene which contain the appropriate regulatory elements, constructing a hybrid plasmid with a marker gene to enable cloned DNA to be introduced into embryos, and monitoring the expression.

Producing monoclonal antibodies to insulin receptors or lipoprotein lipase to inhibit fat uptake by apipocytes is an alternative project.

SOIL SCIENCE

(Vacancy 87/51)

Studies of the retention and subsequent release of nutrient ions by soil and soil components with particular attention to nutrients which are strongly held by soils.

Conditions of Appointment containing further details of these positions are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar, Lincoln College, Canterbury, New Zealand. Applications close 30 November 1987. Please quote appropriate vacancy number when making enquiries.

(W4455)E

UNIVERSITY OF BIRMINGHAM

Department of Biochemistry

POSTDOCTORAL RESEARCH FELLOWSHIPS

Applications are invited for three Research Fellowships each of which is available from 1 January 1988.

Post No.B4562 is for work with Professor A Colman, funded by the Cancer Research Campaign until 28 February 1989, on the translational control and spatial organisation of maternal mRNA in Xenopus oocytes. Techniques employed will include microinjection of nucleic acids and oligonucleotides, in situ hybridization and the use of oocyte-derived cell-free translation systems. Applicants should have a good background in molecular cell biology.

Post No.B4563 is for work with Dr J Davey, funded by the MRC for three years from 1 January 1988 on the molecular control of membrane fusion events. The investigation will involve the use of a cell-free reconstruction system and the characterisation of yeast mutants. Applicants should have a good background in molecular cell biology or yeast genetics.

Post No.B4564 is for work with Dr I P Trayer, funded by the SERC for up to three years, on molecular aspects of muscle contraction and regulation. The investigation concerns the identification of the precise sites of interaction between actin and myosin and involves protein and peptide chemistry and NMR spectroscopy. Applicants should ideally have experience in these techniques.

The appointments will be on the Research Fellow 1A scale (£9,305 to £14,825) plus superannuation according to qualifications. For further particulars telephone 021-472 1301 (ext 2559) quoting reference numbers stated above.

No formal application form. Three copies of application, including full CV and naming three referees to Assistant Registrar (Science), University of Birmingham, PO Box 363, Birmingham B15 2TT by 23 October 1987.

An Equal Opportunities Employer.

ROCKEFELLER FOUNDATION BIOTECHNOLOGY CAREER FELLOWSHIPS

The Rockefeller Foundation announces a program of career development fellowships designed to enable scientists from developing countries, trained at outstanding centers for advanced research in biotechnology, to continue to work at those or other institutions for three months each year, over a period of at least three years, conducting advanced research and keeping abreast of new developments in their fields. The program will focus upon the development and application of advances in molecular and cellular biology and immunology relevant to agriculture, health, and reproductive biology.

Funding will be shared between the Foundation and the host laboratory, with the Foundation providing travel and per diem support. It is hoped that the fellowships will encourate the establishment of ongoing working relationships between outstanding younger scientists working at third world institutions, and research teams at advanced laboratories.

Applicants to this program should have at least Ph.D.- or M.D.-level training, a proven record of scientific productivity, and a permanent position at a research or teaching institution in their home country. A written project proposal must be developed and submitted jointly by the candidate and the laboratory sponsor.

Information about application procedures can be obtained by writing to **Biotechnology Career Fellowships, Fellowship Office, Rockefeller Foundation, 1133 Avenue of the Americas, New York, New York 10036 USA.**
(NW566)E

Joint Fellowship in Molecular Neurobiology between The Lee Research Centre, Laboratory of Neurobiology and Development, National Institute for Medical Research, Mill Hill, London, *and* The Lee International Research Center, Division of Neurosciences, City of Hope National Medical Center, Duarte, California

Postdoctoral Fellowship in Molecular Neurobiology: To study the biological role of defined molecules in neural development and regeneration, particularly as manifest after altered gene expression in transgenic animals. This fellowship is to promote collaboration between The Lee Research Centre (director G. Raisman), Laboratory of Neurobiology and Development at the National Institute for Medical Research in London, and The Lee International Center, Division of Neurosciences, City of Hope, Duarte, California. The fellow will be working in collaboration with Dr Roger Morris and Dr Frank Grosveld (London) and Dr Paul Salvaterra (Duarte). The fellowship will be for an initial 2-year period in London and thereafter by mutual agreement one year extensions in either London or Duarte.

Salary will be according to the salary scales of the host institute, and expenses will be available for required travel between the two institutes.

Applications in triplicate, with CV and names and addresses of two referees to:

North America — Dr Paul Salveterra, Division of Neurosciences, City of Hope Medical Center, 1500 East Duarte Road, Duarte, CA 91010, USA.

Elsewhere — Mr C R Russell, Head of Administration, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, England.
(4229)E

AFRC INSTITUTE OF ANIMAL PHYSIOLOGY & GENETICS RESEARCH CAMBRIDGE

Department of Biochemistry

SHIGEMATSU RESEARCH FELLOWSHIP

Applications are invited from post-doctoral biochemists or cell biologists for a three-year fellowship to work with Drs K D Brown and R B Heap on the role of uterus-derived growth factors in embryonic growth and development during early pregnancy. Emphasis is currently centred on FGF and related heparin-binding polypeptides. A background in protein purification and analysis or in recombinant DNA techniques would be an advantage, but other well-qualified and highly-motivated candidates will be considered. Excellent facilities are available within the Biochemistry Department including cell culture, HPLC, peptide synthesizer and sequencer and oligonucleotide synthesizer. Opportunities will also exist for collaboration with staff of the Department of Molecular Embryology which is housed in the same building.

Starting salary up to £10,622 pa. Applications with full CV and the names of two referees should be sent to Dr K D Brown, AFTC Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT, who will be pleased to provide further information (0223-832312). (4197)E

UNIVERSITY OF GLASGOW

Departments of Chemistry & Genetics

MOLECULAR RECOGNITION IN GENETICALLY-ENGINEERED ENZYMES

Postdoctoral Fellowship

Applications are invited for a postdoctoral position, funded under the SERC Molecular Recognition Initiative, to study the energetics of substrate and inhibitor binding to yeast phosphoglycerate kinase using a combination of site-directed mutagenesis and biophysical chemistry techniques.

The post is available for up to 3 years from 1 January 1988, on Research Assistant scale 1A (salary range £9,865-£11,015).

Recent PhDs in biochemistry, biophysical chemistry, molecular biology, or other relevant area, interested in joining an interdisciplinary research team should send a CV, with names and addresses of at least two academic referees, to: Dr Alan Cooper, Department of Chemistry, Glasgow University, Glasgow G12 8QQ, from whom further details may be obtained (Tel: 041-339 8855, ext 5278 or 6582) (4221)E

THE UNIVERSITY OF WARWICK

Department of Biological Sciences

POSTDOCTORAL FELLOWSHIP IN MOLECULAR BIOLOGY

Applications are invited for a postdoctoral position to investigate the regulation of the expression of glutamine synthetase in Phaseolus vulgaris L (French bean). This work forms part of a larger project, in collaboration with Rothamsted Experimental Station, to understand fully the structure and expression of the four genes encoding this functionally important enzyme in higher plant nitrogen metabolism. The successful applicant will join a small team working on this project at Warwick. The work will involve mRNA and isozyme activity measurements, in situ hybridization, DNA cloning and sequencing, and expression in transgenic plants. Experience of recombinant DNA techniques, not necessarily on plants, is particularly welcome but applicants from other biological disciplines will also be considered. Applicants should have, or should shortly complete, a PhD. The post is available for two years from 1st January, 1988. Salary will be on the Research 1A scale: £9,305 to £15,720 pa.

Additional information and initial enquiries should be addressed to Dr J V Cullimore (telephone 0203 523532 direct line). Application forms from the Executive Officer, Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL quoting Ref: JVC/BS598/O.

An equal opportunities employer. (4225)E

continued on page 2

ASSISTANTSHIPS

UNIVERSITY OF STRATHCLYDE

POSTDOCTORAL ELECTROPHYSIOLOGIST

Applications are invited for a two year Wellcome Trust funded Postdoctoral Research Assistantship in the Department of Physiology and Pharmacology to undertake research into the properties and receptor stimulation in human macrophages. Experience in patch clamping and tissue culture would be an advantage.

Salary: £9,305 to £14,825 pe annum. USS benefit.

Applications (Quote Re R53/87) with full curriculum vita and the names and addresses o three referees should be sent to Professor A L Harvey, Department of Physiology and Pharmacology, University of Strathclyde Glasgow G1 1XQ, from whom fur ther particulars are available Closing date for applications: 1 October 1987. (4185)P

GRANTS AVAILABLE

for
Research on the Resistance of Human Tumors to Chemotherapeutic Agents

Bristol-Myers Company is pleased to announce a $16,000,000 drug resistance research funding program. The goal of this research program is to identify the mechanisms by which human tumors become resistant to chemotherapy and to develop and test methods of overcoming such resistance. Grants will be awarded in two categories. Laboratory grants will be $100,000 per year for five years. Five year grants of $200,000 per year will be awarded for research which directly tests laboratory findings in human trials.

Applications are invited from qualified investigators worldwide. Applicants may be associated with academic institutions, research institutes, government laboratories or other facilities capable of conducting these types of investigations. Applications will not be accepted from individuals affiliated with or employed in the pharmaceutical industry or engaged in for-profit research.

Applications will be accepted until January 1, 1988 and will then be reviewed by a panel of experts, independent of Bristol-Myers. Awards will be announced on or about April 1, with funding commencing July 1, 1988. Additional information about this program can be obtained by writing:

Mr. Gary Doyle
Program Coordinator
Bristol-Myers Company
5 Research Parkway
P.O. Box 5100
Wallingford, CT 06492-7660
U.S.A.

(NW428)H

AWARDS

THE CHARLES H. REVSON CAREER DEVELOPMENT AWARDS PROGRAM AT THE WEIZMANN INSTITUTE OF SCIENCE

Purpose:

To recruit outstanding young faculty members to the Weizmann Institute of Science offering them an award which will secure their salary at the Institute for three years and, provide them as well with a three year research budget of $20,000 a year. The recipients have to qualify for a temporary appointment at the Weizmann Institute of Science at the rank of Senior Scientist (=Assistant Professor). They will be selected on the basis of excellence and the potential to develop into leaders in their field.

Deadline for applications is Dec. 31, 1987. Further information is available on request from: **Academic Secretary's Office, Weizmann Institute, Rehovot 76100, Israel (Bitnet: RSMARJOR at Weizmann, Telex: 381300).** (W4457)N

FELLOWSHIPS cont

Royal College of Surgeons of England

HUNTERIAN INSTITUTE

Department of Dental Sciences

QUINTIN HOGG FELLOWSHIP

Applications are invited for this post, tenable for three years, to work as a postdoctoral research fellow (non-clinical) on markers for oral malignancy. Departmental projects already in progress include the identification of activated oncogenes and differentiation markers in normal, dysplastic and malignant oral mucosa. A close working relationship exists with colleagues in the adjoining Imperial Cancer Research Fund and other laboratories in London.

As it is envisaged that the Quintin Hogg Fellow will work on membrane associated markers expressed during oral malignancy, previous experience with monoclonal antibody production and characterization would be an advantage. However, potential applicants who feel they would contribute to the department's research programme on oral cancer in an alternative area eg, virology, cyto- or molecular genetics are also encouraged to apply.

The post is available immediately. The salary will be in Range 1A (currently £9,305 to £13,675 plus £1,393 London Allowance).

Applications including a curriculum vitae, a full statement of laboratory experience and the names of two referees should be sent to: The Personnel Office, Royal College of Surgeons of England, 35-43 Lincoln's Inn Fields, London WC2A 3PN. Informal enquiries to Professor N W Johnson or Dr S E Chang on 01-405 3474.

Please quote reference 38/87.

Closing date: 29 October 1987

UNIVERSITY OF ABERDEEN

DEPARTMENT OF PLANT SCIENCE

And

UNIVERSITY OF STIRLING

DEPARTMENT OF BIOLOGICAL SCIENCES

RESEARCH IN TROPICAL FOREST ECOLOGY

Applications are invited for a Postdoctoral Research Fellowship to investigate the role of ectomycorrhiza in nutrient cycling and forest dynamics in lowland rain forest within Korup National Park, Cameroon. This is a 3 year project funded by an award to Dr Ian Alexander (Aberdeen) and Dr David Newbery (Stirling) from the Overseas Development Administration. The work will involve analysis of soil nitrogen and phosphorus, isolation of mycorrhizal fungi, and seedling transplant experiments. Applicants should hold a PhD in a relevant discipline.

The appointment will be for 3 years starting as soon as possible. Salary within Range IA Scale £9,305—£11,015 pa.

Further particulars and application forms available from The Personnel Office, The University, Regent Walk, Aberdeen, AB9 1FX (Tel 0224 273500) to whom applications (2 copies) with a full c.v. and letter of application should be returned by 23 October 1987 (WS/037) (4173)F

DEUTSCHES KREBSFORSCHUNGSZENTRUM, HEIDELBERG

INSTITUTE FOR IMMUNOLOGY AND GENETICS

Applications are invited for a **RESEARCH ASSOCIATE** in Tumor Immunology and for **POSTDOCTORAL FELLOWSHIPS**. Studies on active specific immunotherapy of cancer patients, mechanism of *in situ* T cell activation, immunological response monitoring. Clinical cooperation already established within the "Tumorzentrum". We are looking for self-motivated scientists with experience in cancer research (especially metastasis) and cellular immunology. Position of two years (starting January 1988) with possibilities for extension. Salary according to German Federal tariff for senior scientists (BAT 1b) including additional benefits. Applications should include a c.v., list of publications, statement of research interest and two letters of recommendation. Postdoctoral Fellows with special interest in fundamental research on metastasis and immunotherapy in model systems are also invited to apply for a Stipend-Programme.

Please send applications to Prof. V. Schirrmacher, Institut für Immunologie und Genetik, Deutsches Krebsforschungszentrum, D-6900 Heidelberg, FRG. (W4412)E

STUDENTSHIPS

OXFORD UNIVERSITY

Nuffield Department of Surgery

RESEARCH STUDENTSHIP

Applications are invited for a Research Studentship, leading to the Degree of DPhil, investigating the mechanism of antigen-induced tolerance to organ grafts.

The Studentship is tenable for 3 years. Applicants should have a First or Upper Second Class Honours Degree in one of the Biological Sciences.

Applications including a curriculum vitae and the names and addresses of two academic referees should be sent to Dr K Wood, Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU by October 16th, 1987. (4235)F

University of Zurich INSTITUTE OF BIOCHEMISTRY

POSTGRADUATE RESEARCH STUDENTSHIP

Area of research for which applications are invited from graduates with a first or upper second class honours degree in Biochemistry, Biology or Chemistry:

Purification and characterization of low molecular weight growth inhibitors, or structural, chemical and kinetic analysis by cryoenzymological techniques of enzyme-substrate-intermediates with the support of CAMD.

Salary £8,000-10,000 per annum. Applications including a full C.V. and the names and addresses of two referees should be sent as soon as possible to PD Dr. H. Gehring, Institute of Biochemistry, University of Zürich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland. Tel. 1-257 55

UNIVERSITY OF READIN

Department of Horticultur
School of Plant Sciences

SERC CASE STUDENTSHIP

"Early detection of resistance to methyl benzimidazole carbamate (MBC) fungicides"

Applications are invited for three-year SERC CASE Studentship to work with Dr R T V Fox.

The project includes the selectio of monoclonal antibodies raised t tubulins from resistant fungi an the development of a system t detect extremely low levels i populations in the field of glasshouse. The industrial collaborato is Dr B C Baldwin at ICI Plan Protection Division, Jealott's Hi Research Station, where th student will spend periods of on to two months each year to investigate the industrial applications c the research.

The studentship is valued a normal SERC rates with an additional contribution from the Industrial Sponsor.

Applicants should have a Firs or Upper Second Class Honour Degree in Microbiology, or Biological Sciences and preferabl experience of cell culture technique and training in biochemistry Letters of application, accompanied by a full CV and the name of two academic referees, shoul be sent as soon as possible to: D R T V Fox, Department of Horticulture, School of Plant Sciences University of Reading, Building Earley Gate, Reading RG6 2A (4211)F

nature
— the professionals' choice

THE GRADUATE SCHOOL OF BIOLOGICAL SCIENCES
MOUNT SINAI SCHOOL OF MEDICINE
OF THE CITY UNIVERSITY OF NEW YORK

The Mount Sinai School of Medicine of CUNY offers Ph.D and MD/Ph.D. training in eight major subspecialties that encompass cutting edge areas of the biomedical sciences. The research laboratories are located in twelve participating departments as well as three new centers for molecular biology, neurobiology and immunobiology. Some of the aculty research interests include:

- luman Gene Mapping
- lolecular & Biochemical Genetics of Human Diseases
- lolecular biology of DNA & RNA tumor viruses
- enetics and biology of influenza viruses
- olecular mechanism of cellular and viral replication
- olecular mechanism of viral host interaction
- RNA transcription, processing and stability in vivo and in vitro
- ne expression of exocrine gland-specific secretory proteins
- ation and characterization of gene-specific regulatory DNA ding proteins
- nsgenic animals and mammalian gene expression
- crophage and neutrophil physiology
- munological mechanisms of liver diseases
- olecular basis of generation of antibody diversity
- Development and regulation of mammalian lymphoid system
- Molecular biology of plasma membrane receptor
- Pharmacology and molecular biology of neuropeptide processing enzyme
- CNS regulation of cardiovascular function
- Developmental neurobiology of growth factors and their receptors
- Neuroendocrinology of stress and reproduction
- Molecular basis of neurodegenerative diseases
- Neurotransmitter receptor pharmacology isolation and characterization
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continued on page 30

WORKSHOPS

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Further details from:

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Further information may be obtained from: **Professor Dr A. De Leenheer, Laboratoria voor Medische Biochemie en voor Klinische Analyse, Harelbekestraat 72, B-9000 Gent, Belgium. Tel: (091) 21.89.51, ext 324.** (W4468)M

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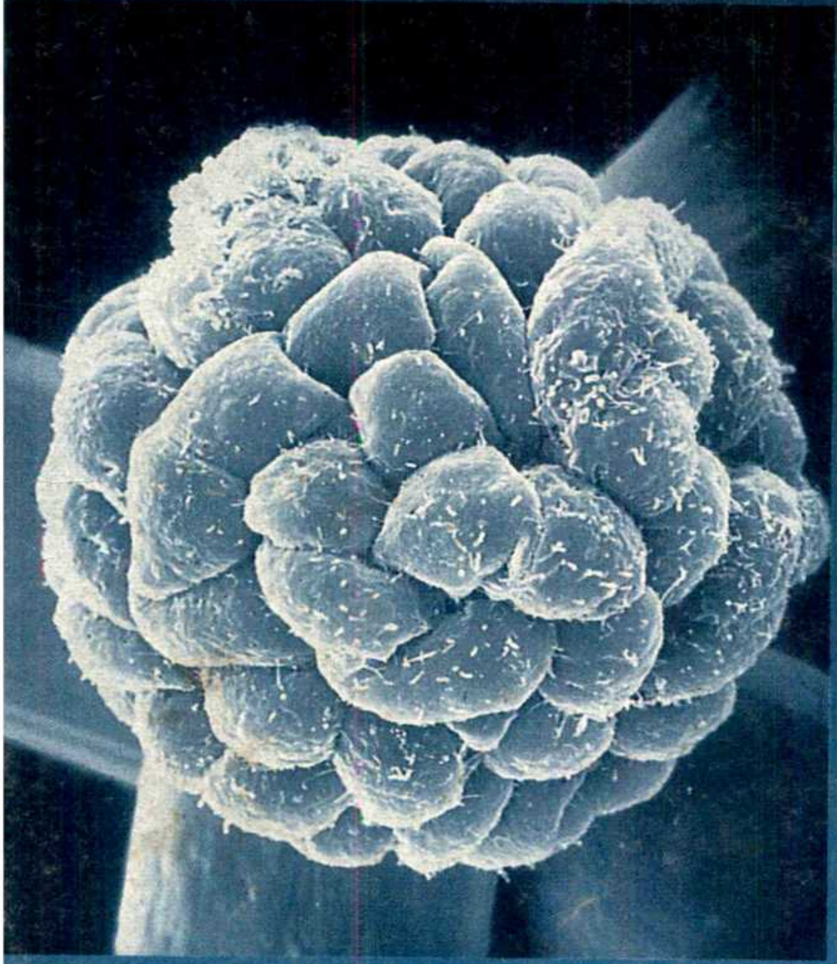

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